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JANUARY–MARCH 2016



Leptonia substricta sp. nov.

(Largent, Kluting, Anderson & Bergemann — PLATE 3, p. 165)

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LORELEI L. NORVELL

editor@mycotaxon.com

Pacific Northwest Mycology Service

6720 NW Skyline Boulevard

Portland, Oregon 97229-1309 USA

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PennycookS@LandcareResearch.co.nz

Manaaki Whenua Landcare Research

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REVIEWERS — VOLUME ONE HUNDRED THIRTY-ONE (1)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

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ERRATA FROM PREVIOUS VOLUMES

VOLUME 130(3)

p.746, line 12 ADD: We also thank Dr. Alan Fryday (Michigan State University, USA) for valuable comments and reviews on the manuscript.

VOLUME 130(4)

p.964, line 10 FOR: 1.5-4 μm diameter READ: 1.5-4 μm diam.

p.964, line 19 FOR: Pegler et. al. 1997 READ: Pegler et al. 1997

p.965, lines 17–18 DELETE:
Yao YJ, Spooner BM. 1995. Notes on British species of *Lamprospora*
and *Ramsbottomia*. Mycological Research 99(12): 1521–1524.
[http://dx.doi.org/10.1016/S0953-7562\(09\)80804-6](http://dx.doi.org/10.1016/S0953-7562(09)80804-6)

p.1038, line 25 FOR: Zhuang WY, Liu XX. READ: Liu XX, Zhuang WY.

FROM THE EDITOR-IN-CHIEF

GUIDE TO ETHICAL RESEARCH NETWORKING — Lucky indeed is the twenty-first century scientist! The rise of academic research network sites (e.g., ResearchGate, Academia.edu, LinkedIn) has recently flooded the Internet with research & conference papers, poster presentations, and databases. Today's mycological researcher has immediate access to a wealth of information that earlier scientists had to search for weeks or months to find.

Much of the information is open and free. MYCOTAXON has long supported open access and is proud of our affiliation with CYBERLIBER, the electronic library for Mycology. Our long-standing agreement allows CYBERLIBER to scan and post all MYCOTAXON papers on its website two years after publication. All mycologists would do well to check the website frequently to access the wealth of mycological literature available there.

However, as Dr. H. Frederick Dylla noted in his letter to the WALL STREET JOURNAL (April 18, Opinion | Letters), "Unless a raw manuscript placed online somehow constitutes the finished product of scientific publishing, "free" can never mean "costless."

And as much as MYCOTAXON would love to publish an entirely free & open access journal, it is our modest page charges and subscription fees that make it possible for us to publish well formatted and thoroughly reviewed mycological research papers. Our dependence upon subscriptions means that unless our authors have paid the optional open access fee, they should not post their papers on the Internet until two years after publication.

Nonetheless, Mycotaxon DOES recommend (perhaps even URGE) its authors to announce new papers by submitting title, abstract, and DOI number to their favorite research network as soon as their work has been published. Informing these networks of your recent accomplishments only makes sense!

MYCOTAXON 131(1) covers 28 papers by 101 authors (representing 17 countries) and revised by 51 expert reviewers.

Within its pages are three new genera (*Anacoronospora* and *Dictyoaquaphila* from Brazil, and *Sympodiosynnema* from China) and 33 species new to science representing *Leptonia* from Australia; *Anacoronospora*, *Dictyoaquaphila*, *Mirandina*, *Pleurothecium*, and *Sporidesmiopsis* from Brazil; *Gibellula*, *Sarcogyne*, and *Sympodiosynnema* from China; *Rhodocollybia* from Costa Rica, Dominican Republic, and Puerto Rico; *Passalora* from India; *Ramularia* from Korea; *Annulohypoxylon*, *Aspergillus*, and *Ustulina* from Thailand; and *Fomitiporia* and *Puccinia* from the U.S.A.

In addition to range extensions and/or new hosts for previously named taxa, we also offer new combinations in *Agaricus* and *Annulohypoxylon*.

2016 brings additional changes: A full color photo now graces our front cover, while an original drawing graces the back cover. Our nomenclatural novelties and typifications page now also presents the nomenclatural identification number after each new name.

Warm regards,

Lorelei Norvell (*Editor-in-Chief*)

20 April 2016

PUBLICATION DATE FOR VOLUME ONE HUNDRED THIRTY (4)

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FOUR STEPS TO SUCCESSFUL MYCOTAXON PUBLICATION IN 2016

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3—FINAL SUBMISSION: Consult experts, revise and thoroughly proof manuscripts, and prepare error-free text and image files ready for immediate publication. Label the Email **AND ALL FILES** with the accession number before attaching the (i) 2016 MYCOTAXON submission form; (ii) text files for main text, tables, and legends; (iii) final art files to the *Editor-in-Chief* <editor@mycotaxon.com>, and (iv—NEW!) nomenclatural identification verification for each new name. The *Editor-in-Chief* usually Emails all coauthors and expert reviewers within two weeks of final submission, but please wait at least 14 days before sending a follow-up query (without attachments).

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New hosts for *Bartalinia* and *Chaetopyrena* in China

YAN WANG¹, LING JIN^{1*}, LI LIN¹, TIAN-TIAN ZHU¹,
XIU-RONG CHEN² & LI-PING CHAO³

¹ Gansu University of Traditional Chinese Medicine, Lanzhou 730000, China

² College of Pratacultural Science & Key Laboratory of Grassland Ecosystem,
Gansu Agricultural University, Ministry of Education

Sino-U.S. Center for Grazingland Ecosystem Sustainability, Lanzhou 730070, China

³ Tianjin University of Traditional Chinese Medicine, Tianjin 300100, China

*CORRESPONDENCE TO: zyxyjl@163.com

ABSTRACT—Two anamorphic species, *Bartalinia robillardoides* and *Chaetopyrena penicillata*, are reported on a new host in China. Both endophytic fungi were isolated from stems of *Ephedra intermedia* and are described and illustrated.

KEY WORDS—*Amphisphaeriaceae*, appendaged coelomycetes, *Didymellaceae*, endophytes, taxonomy

Introduction

In the course of investigating the endophytic fungi of *Ephedra intermedia* in Gansu province, China, healthy stems were surface-sterilized in 1% sodium hypochlorite for 1 minute and then rinsed three times in sterilized distilled water, and tissues were plated onto PDA to obtain pure cultures. Two coelomycetous fungi were isolated. Based on their cultural characteristics, morphological comparison, and analysis of the internal transcribed spacer region of ribosomal DNA (rDNA-ITS), the fungi were determined as two coelomycetous species on a new host in China. *Chaetopyrena penicillata* is reported for the first time from China.

Materials & methods

Isolates and morphology

The morphology of the strains was examined on OA: oatmeal agar (30 g oatmeal, 1000 mL distilled water), MEA: malt extract agar (15 g malt extract, 1 g peptone, 20 g

dextrose, 15 agar, 1000 mL distilled water) and PDA: potato dextrose agar (200 g potato, 20 g dextrose, 15 g agar, 1000 mL distilled water), incubated for 10 d at 25°C in the dark. All structures were described from 1-month-old PDA cultures.

Specimens are deposited in the Herbarium of Gansu University of Traditional Chinese Medicine, Lanzhou, China (HTCM).

DNA extraction and sequencing of (rDNA-ITS)

Mycelium was scraped from cultures growing on PDA, put into 100 mL PD, agitated using an orbital shaker set at 45 r.p.m. and incubated at room temperature under constant illumination by fluorescent lights. Mycelium (100 mg) was removed after 7 days and transferred to a mortar and pestle for grinding with liquid nitrogen. DNA extraction was then carried out using the FastDNAkit (SK1375, Sangon Biotech Co. Ltd.) following the manufacturer's instructions. The presence of DNA was verified by separation on a 0.8% agarose gel in 1×TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) stained with ethidium bromide (0.5 mg/L) and visualized under UV light. The ITS region was amplified using the universal primers, ITS1 and ITS4 (White et al. 1990). PCR was performed in 50 µL reaction volumes containing 10–100 ng of genomic DNA, 2.5 µL of each primer (10 µM), 0.25 µL dNTP (10 mM), 2.0 unit Supertaq DNA polymerase and 5 µL 10 × PCR buffer (Sangon Biotech Co. Ltd.). The amplification was performed in a thermocycler (Biometra Tg PCR). The PCR thermal cycle program was as follows: 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 3 min, annealing at 52°C for 45 s, and elongation at 72°C for 1 min, with a final extension step of 72°C for 8 min. The PCR products, spanning approximately 500 bp of ITS, were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The PCR product was purified and sequenced using the above-mentioned primers by Sangon. Approximate phylogenetic placements were determined with the Blastn search option of the National Center for Biotechnology Information (NCBI) and the suggested identities are discussed in the descriptive notes below.

Taxonomy

Bartalinia robillardoides Tassi, Bull. Lab. Orto Bot. Reale Univ. Siena 3: 5. 1900.

PLATE 1

Black conidiomata in PDA culture, stromatic, globose or subglobose, immersed or semi-immersed, solitary, glabrous, 605–757 × 585–702 µm diam, black to brownish black, wall thick, composed of bright brown, thick-walled cells, paler and thinner in inner layers. Ostiole absent, dehiscence irregular by breakdown of upper wall. Conidiophores absent. Conidiogenous cells cylindrical to subcylindrical, hyaline, 6–9 × 3 µm. Conidia cylindrical or fusiform, smooth-walled, straight to slightly curved, 4-septate, slightly constricted at septa, hyaline, 25.6–33.3 × 2.6–3.1 µm, length/width ratio 9.9. Apical cell hyaline, conic, thin-walled, 3.0 µm long, bearing (2–)3(–4) appendages from apex. Appendage branches 2–3, divergent, flexible, tapering gradually towards apex, 11.5–19.8 µm long. Median cells cylindrical, ochraceous

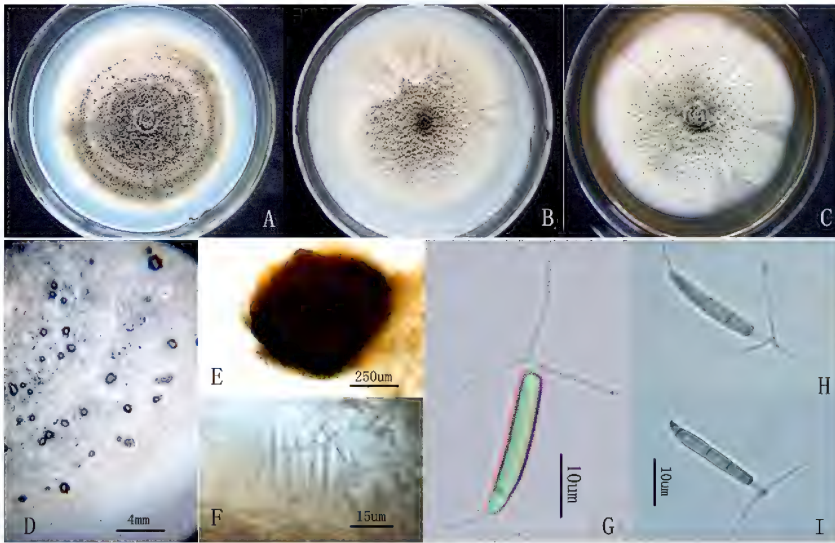


PLATE 1. *Bartalinia robillardoides* (HTCM 1009024). A–C: Ten-day-old colonies on PDA (A), OA (B), and MEA (C); D, E: Conidiomata; F: Conidiogenous cells with developing conidia; G–I: Conidia.

to pale brown; the cell next to basal cell longest, 7.7–11.5(–14.1) μm (mean = 10.1 μm), $l : w = 3$. Basal appendage straight, (5.1–)7.7–9.5(–10.3) μm long (mean = 8.5 μm). Colony on PDA at 25°C grayish green, regular, with darker concentric zones, aerial mycelium floccose, slightly grey black in reverse, producing numerous black conidiomata, growth rate 7.3 mm/d. Colony on OA grayish green, no concentric zones, the reverse grey, producing sparse brown conidiomata, growth rate 6.5 mm/d. Colonies on MEA white to grayish white, sparse conidiomata, growth rate 6.3 mm/d.

SPECIMEN EXAMINED: CHINA: GANSU PROVINCE, Lanzhou city, Renshou mountain, isolated from stems of *Ephedra intermedia* Schrenk & C.A. Mey. (*Ephedraceae*), 20 Sep, 2009, Yan Wang. (HTCM 1009024; dried cultures, HTCM–F: 09122, 09123; GenBank, KF656706).

COMMENTS — *Bartalinia robillardoides* is reported from diverse hosts around the world, including Hong Kong, China (Zhuang 2001, as *Seimatosporium robillardoides*), but it has not previously been reported from *Ephedra intermedia*. The identification of this isolate was made based on conidial morphology as well as on molecular sequence data. The Blastn search results of the ITS sequence of our Chinese isolate from *Ephedra* (GenBank KF656706) showed 99% similarity to *B. robillardoides* (GenBank EU552102), 100% similarity to

Ellurema sp. (GenBank AY148442), 96 and 95% similarity to two isolates of *B. pondoensis* (GenBank GU291796, AF405302), and 95% similarity to *B. laurina* (GenBank AF405302).

Nag Raj (1993), who published a comprehensive account of *Bartalinia*, accepted and keyed six species and listed another nine species as “unexamined and excluded taxa.” Andrianova & Minter (2007) accepted and keyed ten species were by, and Marincowitz et al. (2010) added a further species. Based on Nag Raj (1993), our *Ephedra* isolate keys to *B. robillardoides* based on its size and 4-septate conidia. Andrianova & Minter (2007) emphasized the importance of even or uneven conidial septation, conidial size, and number of appendage branches; in their key, our *Ephedra* isolate again keys to *B. robillardoides* because of the large cell next to the base, conidial size, and generally 3-branched apical appendage. The most recently described species, *B. pondoensis*, is unlike *B. robillardoides* in having 3-septate conidia. The genus *Ellurema* is applied to the sexual state of *Hyalotiopsis*, a genus that is distinguished from *Bartalinia* by characteristics of the conidial appendages (Nag Raj 1993).

Chaetopyrena penicillata (Fuckel) Höhn., Hedwigia 60: 132, 1918. PLATE 2

Conidiomata superficial. Pycnidia single, solitary, globose to subglobose, dark brown to black, ostiolate, 320–400 µm diam, surrounded by setae, neck 100 × 50 µm; setae brown, thick-walled, septate, unbranched, gradually tapering upward, up to 150–180 µm long; wall consisting of 3–4 layers of brown textura angularis. Conidiophores hyaline, simple. Conidia hyaline, smooth, 1-celled, cylindrical to sub-cylindrical, straight, with rounded apex and truncate base, 12.6–17.7 × 2.5–5.1 µm (mean = 15.9 × 3.9 µm). Colonies on PDA 42 mm diam. after 7 days at 25°C in dark, smooth, black, margins grayish white, regular, reverse black, aerial mycelium sparse. Colonies on OA 36 mm diam after 7 days at 25°C in dark, substrate mycelium orange-green. Colonies on MEA reaching 30 mm diam. after 7 days at 25°C in dark; aerial mycelium grayish white; pycnidia not formed; chlamydospores not seen.

SPECIMEN EXAMINED: CHINA: GANSU PROVINCE, Lanzhou city, Renshou mountain, isolated from stems of *Ephedra intermedia*, 1 Oct. 2009, Yan Wang. (HTCM 1009028; dried cultures, HTCM-F: 09124, 09125; GenBank, KC492443).

COMMENTS — A Blastn search of the ITS sequence of our Chinese isolate from *Ephedra* (GenBank KC492443) showed 99% similarity to *Chaetopyrena penicillata* (GenBank JQ663990). Our isolate agrees morphologically with this species as described in Arzanlou & Khodaei (2012). This genus is currently placed in the *Didymellaceae* (Gruyter et al. 2010). Although 19 species have been placed in *Chaetopyrena*, most species are obscure and do not have living cultures. Arzanlou & Khodaei (2012) reported *C. penicillata* as the cause of

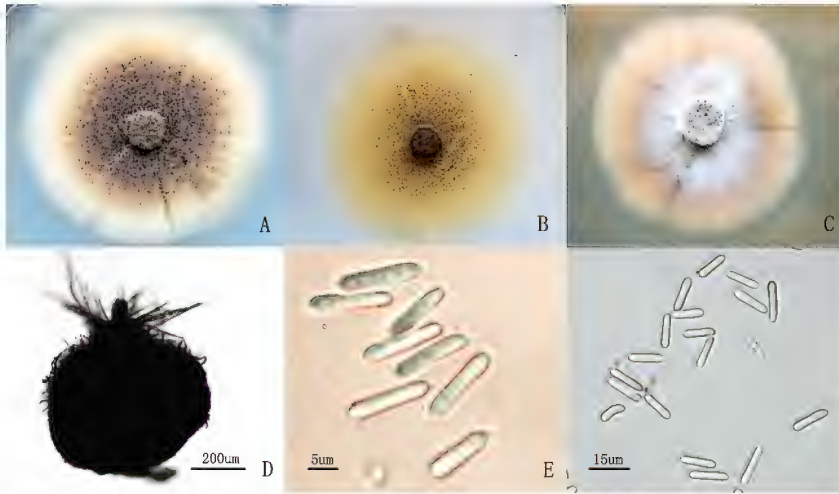


PLATE 2. *Chaetopyrena penicillata* (HTCM 1009028). A–C: Ten-day-old colonies on PDA (A), OA (B), and MEA (C); D: Conidiomata; E, F: Conidia.

dry rot on Russian olive (*Elaeagnus angustifolia*). It is also reported from soil as well as dead twigs, fruit, and stubble in the Middle East, Russia, and South Africa. This is the first report of *C. penicillata* from China, and the first report from *Ephedra*.

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The authors are grateful to Amy Rossman (Systematic Mycology & Microbiology Laboratory, USDA-ARS), Keiichi Motohashi (Electron Microscope Center, Tokyo University of Agriculture), and Lorelei Norvell (Mycotaxon Editor-in-Chief) for critical comments, corrections, and pre-submission reviews. This study has been supported by National Nature Science Foundation, project 31460013, Gansu Nature Science Foundation, project 1212RJZA092, Gansu College Scientific Research, project 2013A-089 and Gansu Herbal Medicine Industry Research Foundation, project GYC11-01.

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***Aspergillus similanensis* sp. nov. from a marine sponge in Thailand**

TIDA DETHOUP^{1*}, NELSON GONÇALO MORTÁGUA GOMES²,
SRIMEK CHAOPONGPANG¹ & ANAKE KIJJOA²

¹ Department of Plant Pathology, Faculty of Agriculture, Kasetsart University,
50 Ngam Wong Wan Road, Chatuchak, Bangkok, 10900, Thailand

² ICBAS-Instituto de Ciências Biomédicas Abel Salazar and Interdisciplinary Centre of Marine &
Environmental Research (CIIMAR), Universidade do Porto,
Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

* CORRESPONDENCE TO: agrtdd@ku.ac.th

ABSTRACT —A new species of *Aspergillus* sect. *Fumigati*, *A. similanensis*, isolated from the marine sponge *Rhabderrmia* sp. collected from a coral reef of the Similan Island, Phangnga province, Thailand, is described and illustrated. *Aspergillus similanensis* is characterized by production of finely reticulate ascospores with two low equatorial crests, a yellowish colony on malt extract agar, and extremely restricted growth on Czapek agar. The validation of this new species is further supported by sequence analyses of the β -tubulin, calmodulin, and actin genes.

KEY WORDS — sponge-associated fungi, phylogeny, taxonomy

Introduction

Aspergillus sect. *Fumigati* W. Gams et al. [= *Neosartorya* Malloch & Cain] is widely distributed, being reported mainly from soil, debris, and foods (Samson et al. 2007, Matsuzawa et al. 2014a,b) but recently also from marine environments, such as from marine sponges (Eamvijarn et al. 2013a). Despite the reports on their pathogenicity to humans and animals (Farrell et al. 2014, Howard 2014), *Neosartorya* species are also prolific sources of secondary metabolites with a wide range of biological activities (Eamvijarn et al. 2013b; Gomes et al. 2014; Prompanya et al. 2014, 2015).

Samson et al. (2007) revised the taxonomy of *Aspergillus* sect. *Fumigati* and *Neosartorya* based on phenotypic and molecular characteristics, describing 33 species, including 23 with teleomorphic states. Since then, several new species in *A.* sect. *Fumigati* have been reported: Eamvijarn et al. (2013b) described *A. siamensis* from coastal forest soil in Samaesarn Island, Thailand; Hubka et al. (2013) described *A. waksmanii* from New Jersey soil and *A. marvanovae* from contaminated water in the Czech Republic; Matsuzawa et al. (2014a,b) described *A. caatingaensis* and *A. pernambucoensis* from semi-desert soil in Brazil and *A. huiyanaiae* from desert soil in Xinjiang, China; and Nováková et al. (2014) described *A. brevistipitatus*, *A. conversis*, and *A. wyomingensis* from reclamation site soils in Wyoming, USA.

In our ongoing search for bioactive compounds from fungi against plant pathogens, unusual isolates of *Aspergillus* sect. *Fumigati* have been isolated from the marine sponge *Rhabdermia* sp. Here, we describe and illustrate the sponge isolates as a new species, *A. similanensis*.

Materials & methods

Fungal Isolation'

The marine sponge *Rhabdermia* sp. was collected at a coral reef in the Similan Islands National Park (8°39'09"N 97°38'27"E), Phangnga province, Thailand. The sponge tissue was washed three times with sterile sea water, cut into 0.5 × 0.5 cm pieces, and placed on half potato dextrose agar (PDA) with 70% of sea water and 300 mg L⁻¹ of streptomycin sulphate. The plates were incubated at 28°C for 7 days.

Hyphal tips from a pure fungal strain were transferred onto PDA slants and stored in the Culture Collection, Department of Plant Pathology, Kasetsart University, Bangkok, Thailand (KUFA) for subsequent identification and deposited in a metabolically inactive state at BIOTEC Culture Collection, Pathumthani, Thailand (BCC).

Morphological Identification

Macroscopic characteristics were observed from the colonial growth pattern (i.e., color and texture) on malt extract agar (MEA), cornmeal agar (CM), Czapek agar (CzA), oatmeal agar (OA), and Czapek yeast extract agar (CYA), after incubation at 25°C, 37°C, and 45°C for 7 days (Samson et al. 2007). Colony colors were designated according to Rayner (1970). After incubation, microscopic characteristics were thoroughly examined under a light microscope in a slide preparation using sterile distilled water as the mounting medium. Ascospore ornamentation was examined by scanning electron microscope (SEM: JEOL JSM 6400).

DNA extraction and molecular Identification

DNA was extracted from young mycelia following a modified Murray & Thompson (1980) method. Primer pairs Bt2a and Bt2b were used for β -tubulin gene amplification (Glass & Donaldson 1995), cmd5 and cmd6 for calmodulin gene amplification (Hong et al. 2005), and ACT-512F and ACT-783R for actin gene amplification (Carbone & Kohn 1999). The PCR products were sequenced using dideoxynucleotide chain

termination method (Sanger et al. 1977) (Macrogen Inc., South Korea). DNA sequences were edited using Finch TV software. The gene sequences were aligned and phylogenetic trees were generated using Clustal X software (Thompson et al. 1997) utilizing neighbor-joining analysis (Saitou & Nei 1987). The distances between sequences were calculated based on the two-parameter model by Kimura (1980). A bootstrap was conducted with 1000 replications (Felsenstein 1985).

Taxonomy

Aspergillus similanensis Dethoup, sp. nov.

FIGS 1–4

MYCOBANK MB810782

“*Aspergillus similanensis*” Prompanya et al., Mar. Drugs 12: 5160. 2014, nom. nud.

“*Aspergillus similanensis*” Prompanya et al., Mar. Drugs 13: 1342. 2015, nom. nud.

Differs from other *Aspergillus* species by its finely reticulate ascospores with two low equatorial crests, the production of a yellowish colony on MEA, and extremely restricted growth on CzA.

TYPE: Thailand, Phangnga Province, Similan Island, a dried culture from marine sponge, *Rhabdermia* sp., collected from a coral reef, April 2010, T. Dethoup (**Holotype**, BCC 75436; ex-type cultures KUFA 0012, KUFA 0013; GenBank KM095494, KC920701, KM202074).

ETYMOLOGY: Referring to Similan Island where the sponge was collected for fungal isolation.

Colonies on MEA growing rapidly, attaining a diameter of 35 mm in 7 days at 25°C, consisting of a floccose mycelium, producing abundant ascomata on the mycelial felt, white to creamish, margins broad, thin and plane; conidiogenesis absent; exudate none; odor lacking; reverse luteous (R12).

Colonies on CzA growing extremely restrictedly, attaining a diameter of 8–10 mm in 14 days at 25°C, producing only white mycelium covering the mycelial plug. Colonies on CYA attaining a diameter of 15–20 mm in 7 days at 25°C, velvety, floccose at the central, consisting of a compact basal felt, furrowed, margins irregular, pale luteous (R11) to sulphur yellow (R15); ascomata and conidiogenesis absent; exudates and soluble pigment absent; reverse wrinkle, slightly zonate, rust (R39).

Colonies on CM spreading broadly, attaining a diameter of 35 mm in 7 days at 25°C, plane, velvety, consisting of thin mycelial felt, producing abundant developing ascomata over the entire surface, margins thin and submerged, white in color; conidiogenesis lacking; reverse luteous (R12).

Colonies on OM showing the same colonial characteristic as on CM, attaining a diameter of 30 mm in 7 days at 25°C, plane, velvety, consisting of thin mycelial felt, producing abundant developing ascomata at the central colony, margins thin and submerged, white in color; conidiogenesis lacking; reverse luteous (R12).

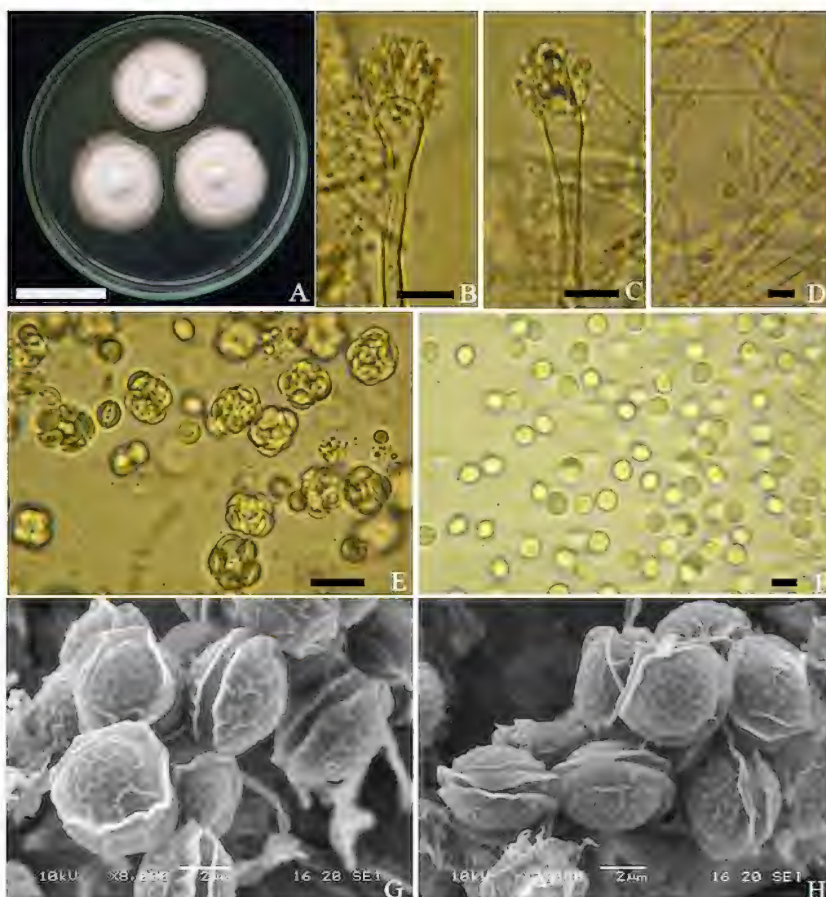


FIG. 1. *Aspergillus similanensis* (KUFA 0012): A. colony on MEA 25°C, 7d; B, C. conidial heads; D. conidia; E. asci; F. ascospores; G, H. ascospores (SEM). Scale bars: A = 3 cm; B, C, E = 10 μm, D, F = 5 μm.

Homothallic.

At 37°C, colonies on all media except CzA growing more rapidly than at 25°C, but no growth at 45°C.

Mycelium composed of branched, septate, smooth-walled, hyaline hyphae, 2–3 μm wide. Conidial heads small, short radiate, conidiophores 120–170 × 3–4 μm wide in the middle, thick and smooth-walled, frequently septate at basal area, vesicles subglobose, pyriform or subclavate 7–8 μm wide in diameter; uniseriate, phialides hyaline, 6–7 × 2–2.5 μm, covering the upper half of the vesicle, conidia pale green, globose to subglobose, 3–4 μm, smooth walled.

Ascomata non-ostiolate, superficial, globose, 120–150 µm, ripening within 10–14 days. Covering consisting of loosely hyaline, 4–5 µm wide, branched, septate, smooth-walled hyphae. Asci 8-spored, globose to subglobose, 10–11 µm, evanescent at maturity. Ascospores hyaline, lenticular, 5–5.5 µm including two low equatorial crests, 1–1.5 µm wide, spore body 4–4.5 µm and with the convex surfaces fine reticulate ornamentation.

COMMENTS—The strain KUFA 0012 produced lenticular ascospores, convex surfaces decorated with fine reticulation with two low equatorial crests (FIGS 1G–H). Ascospores of this species are similar to those of *A. huiyanaiae* and *A. pernambucoensis*, which were recently reported from soils. In both of those species the convex surface ornamentation of the ascospores is irregularly ribbed to slightly reticulate. However, strain KUFA 0012 can be distinguished from the two other species by its smaller ascospore size as well as colony morphology on CzA in term of sporulation rate, colony coloration, and growth rate. Phylogenetic analysis of the β -tubulin gene comparing KUFA 0012 and 43 other strains for comparison (Matsuzawa et al. 2014b) and the calmodulin gene comparing KUFA 0012 with 10 related species revealed a close relationship with *Aspergillus huiyanaiae* (FIGS 2–3), while actin gene analysis placed KUFA 0012 in the same clade with *N. fennelliae* and *N. sublevispora* (FIG. 4). The β -tubulin, calmodulin, and actin DNA sequences were additionally analyzed using BLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The β -tubulin gene analysis showed KUFA 0012 closely related (98%) to *N. fennelliae*, and the calmodulin and actin gene analyses revealed a 97% of similarity with *N. fennelliae*.

Our phylogenetic analysis placed KUFA 0012 in a clade closely related to *A. huiyanaiae*, *N. fennelliae*, and *N. sublevispora*. Despite its morphologically similar ascospores with *A. huiyanaiae*, the KUFA 0012 strain produces smaller ascospores with two low equatorial crests. KUFA 0012 also differs from *N. sublevispora* in its distinct ascospore ornamentation presenting finely reticulate ascospores with two well separated crests instead of regularly microtuberculate ascospores with two closely appressed crests. Although the gene analysis revealed a close phylogenetic relationship with *N. fennelliae*, the strains are clearly separated by mating type: KUFA 0012 strain is homothallic and *N. fennelliae* was described as heterothallic.

Phylogenetic and morphological analyses showed that the marine-derived taxon is an undescribed species, which we have named *Aspergillus similanensis*. As recently reported by our research group (Prompanya et al. 2014, 2015), the ethyl acetate extract from KUFA 0013 contains seven new natural products—chevalone E; pyripyropene E and T; similanamide; and similanpyrone A, B,

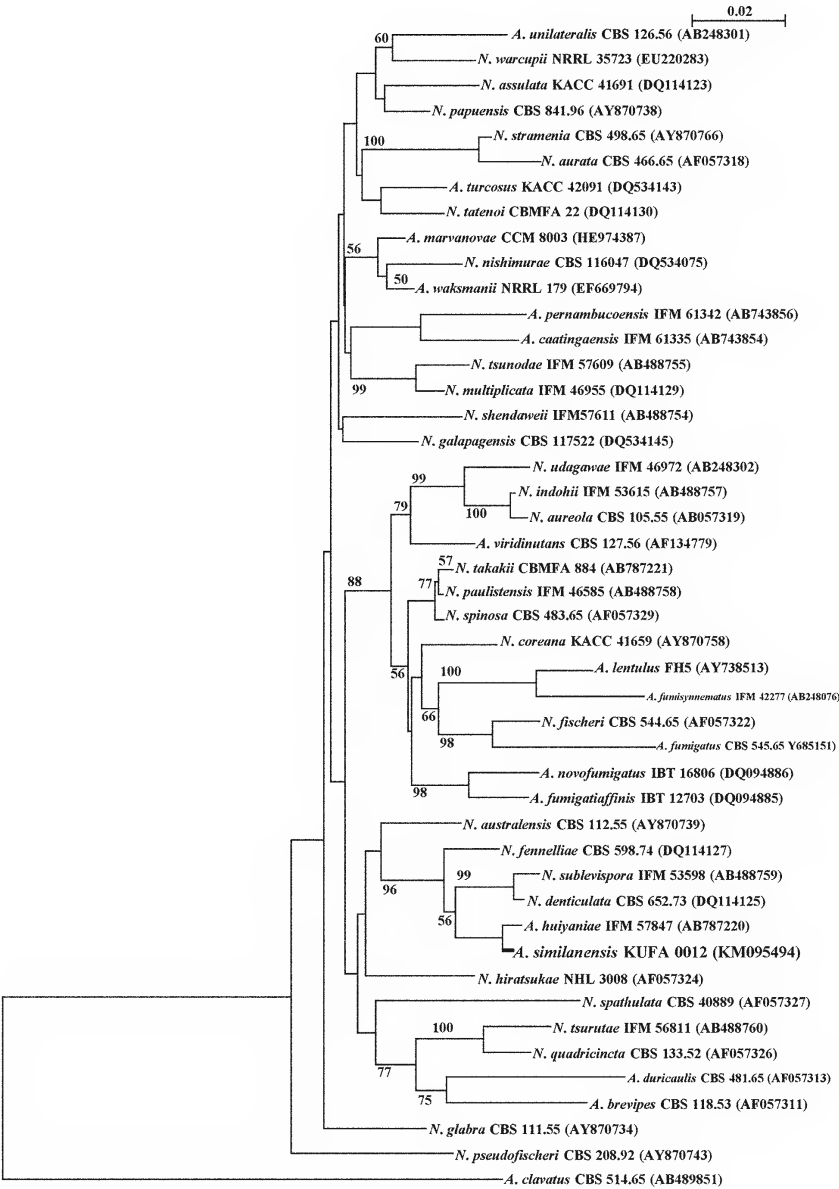


FIG. 2. Neighbor-joining tree from sequences of the β -tubulin gene. Numbers at nodes indicate the percentage of bootstrap sampling (>50%), derived from 1000 samples. *A.* = *Aspergillus*, *N.* = *Neosartorya*.

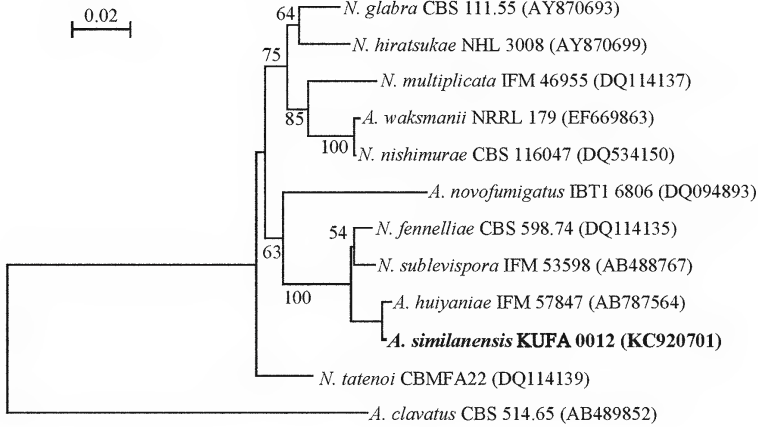


FIG. 3. Neighbor-joining tree from sequences of the calmodulin gene. Numbers at nodes indicate the percentage of bootstrap sampling (>50%), derived from 1000 samples. *A.* = *Aspergillus*, *N.* = *Neosartorya*.

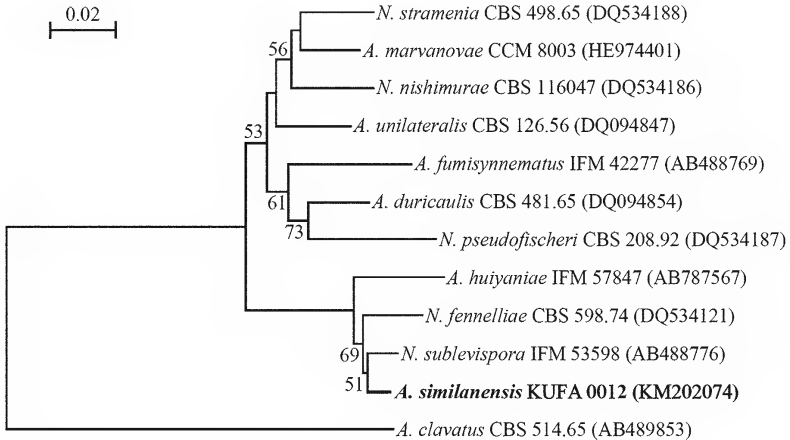


FIG. 4. Neighbor-joining tree from sequences of the actin gene. Numbers at nodes indicate the percentage of bootstrap sampling (>50%), derived from 1000 samples. *A.* = *Aspergillus*, *N.* = *Neosartorya*.

and C—as well as seven previously described compounds. Interestingly, this new species was isolated from a marine sponge, an unusual habitat for teleomorphic states of *Aspergillus* sect. *Fumigati*, which have mainly been reported from soils and food (Eamvijarn et al. 2013a, Matsuzawa et al. 2014a,b). Despite the limited distribution in marine environments, we have previously reported several species of *Neosartorya* such as *N. paulistensis*, *N. laciniosa*, and *N. tsunodae* from marine sponges collected from Thai waters (Gomes et al. 2014). Chemical analysis of these species resulted in the isolation of several bioactive compounds (Eamvijarn et al. 2013a, Gomes et al. 2014). Since we have found only one strain of *A. similanensis*, we consider this species rare and presumably with limited distribution.

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First record of *Mycena plumipes* in Romania, with notes on its presence on pine cones

VASILICĂ CLAUDIU CHINAN* & LUCIAN FUSU

Faculty of Biology, Alexandru Ioan Cuza University,
Iași, Bd. Carol I, No. 20A, 700505, Romania

*CORRESPONDENCE TO: vasilechinan@yahoo.com

ABSTRACT — *Mycena plumipes* was found in Romania on fallen and partially buried cones of *Pinus nigra* and *Picea abies*. The analysis of ribosomal DNA internal transcribed spacer sequences showed that the specimens collected in Romania on *P. nigra* belong to *M. plumipes*. This is the first record of *M. plumipes* in Romania and confirms that it grows on pine cones (as well as spruce cones), based on molecular and morphological data. A detailed description and illustrations of macroscopic and microscopic aspects of the Romanian specimens are given.

KEY WORDS — Basidiomycota, *Mycena* sect. *Fragilipedes*, *Mycena strobilicola*, habitat

Introduction

Mycena plumipes is a vernal, cone-inhabiting species that belongs to *M.* sect. *Fragilipedes* (Fr.) Quél. (Robich 2006). It was described by Kalchbrenner (1873) under the name “*Agaricus Collybia plumipes*” [i.e., *Agaricus (Collybia) plumipes*]. In his original description, Kalchbrenner did not mention its habitat very precisely, stating the substrate as “ad conos pinuum” (i.e., “on pine cones”), but in the lectotype figure the basidiomata are illustrated on what appears to be a *Picea* cone (rather than a *Pinus* cone). In addition, Kalchbrenner mentioned that *Agaricus plumipes* is accompanied in its habitat by *Agaricus esculentus* Wulfen [≡ *Strobilurus esculentus* (Wulfen) Singer], a species that grows in spring on *Picea* cones (Gulden 1966). Moreau (2003) evaluated these unclear aspects to conclude, based on the iconography and accompanying species, that a cone very similar to one of *Picea* serves as the substrate of *Agaricus plumipes*. Also, Moreau showed that *Agaricus plumipes* is an earlier synonym of *Mycena*

strobilicola J. Favre & Kühner, which also was described from *Picea* cones (Kühner 1938), and went on to propose the combination *Mycena plumipes*.

In Romania, this species was found on fallen cones in plantations of both *Pinus nigra* J.F. Arnold and *Picea abies* (L.) H. Karst. Here we report the first records of *M. plumipes* in Romania and demonstrate the conspecificity of the specimens collected from cones of Norway spruce and black pine using molecular markers.

Materials & methods

Specimens of *Mycena plumipes* were collected in April 2011 and 2012 from fallen and partially buried cones of *Pinus nigra* and *Picea abies*. Fragments from fresh and dried basidiomata were mounted in Congo Red and Melzer's reagent for examination under the light microscope (LM). The specimens were identified using Breitenbach & Kränzlin (1991), Kühner (1938), Elborne et al. (1992), Emmett et al. (2008), Maas Geesteranus (1988), Moreau (2003), Robich (2003, 2006), and Roux (2006). The collected basidiomata were dried and deposited in the Herbarium of the Alexandru Ioan Cuza University, Iași, Romania (I). The description is based on the Romanian specimens collected by the authors.

In order to verify that specimens growing on *Pinus* and *Picea* cones are conspecific (and to confirm the species identity), we targeted the nuclear ribosomal internal transcribed spacer (ITS) region, recently formally proposed as the primary fungal barcode marker (Schoch et al. 2012). The ITS DNA sequences were generated from dried basidiomata using primers ITS1F 5'-CTGGTCATTAGAGGAAGTAA-3' (Gardes & Bruns 1993) and ITS4, 5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990) and sequenced at a commercial facility (Alvalab, Santander, Spain). The obtained ITS sequences are deposited in GenBank (accession numbers JX297424–297427). Sequences of *Mycena* sect. *Fragilipedes* retrieved from GenBank were aligned with the ClustalW algorithm (Larkin et al. 2007) with a gap opening penalty of 10 and a gap extension penalty of 1. The most appropriate substitution model was selected using a Maximum Likelihood (ML) criterion. The ML analysis with 1000 bootstrap replicates was conducted using the Tamura 3-parameter model with a discrete Gamma distribution (T92 + G) indicated by BIC as the best-fit model. Nucleotide-sequence divergences were calculated using the K2P model (Nei & Kumar 2000). All phylogenetic analyses were performed using MEGA 6.0.5 (Tamura et al. 2013).

Taxonomy

Mycena plumipes (Kalchbr.) P.-A. Moreau, Bull. Mycol. Bot. Dauphiné-Savoie 43(171): 5 (2003)

PLATE 1

Basidiomata 40–70 mm high. Pileus 15–30 mm diam., hemispherical, campanulate or conical, with a broad or truncate umbo; finely fibrillose under the lens, hygrophanous, sometimes finely translucently striate at the edge; the colour variable: brown, light brown, ochraceous brown, or greyish brown, margin concolorous or paler. Lamellae 26–35 reaching the stipe (with 3–5

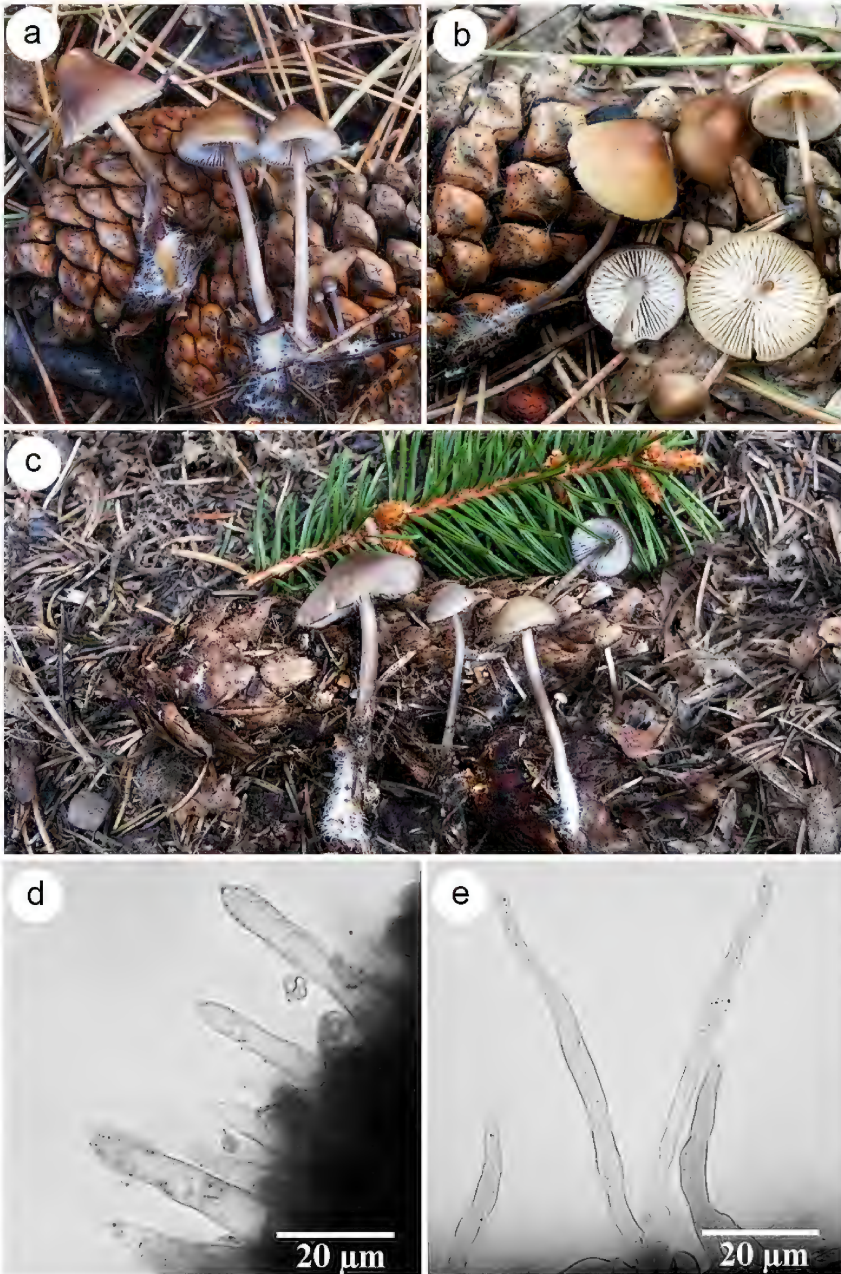


PLATE 1. *Mycena plumipes*: a, b. basidiomata on *Pinus nigra* cones; c. basidiomata on *Picea abies* cone; d. cheilocystidia (LM); e. caulocystidia (LM)

intervening lamellulae), ventricose, adnate-emarginate with decurrent tooth, ≤ 5 mm broad, white, whitish to pale grey, turning to pink with age, edge entire. Spore deposit white. Stipe 30–70 $\times$ 2–3 mm, central, cylindrical, straight to slightly curved, equal, hollow, glabrous, grey brown, apex paler to whitish and minutely pruinose, base at the contact zone with the cone covered with coarse, long, whitish fibrils. Context whitish to pale grey. Odor nitrous.

Basidia 25–30 $\times$ 6.5–7.5 μm , 4-spored. Basidiospores 7–10 $\times$ 4–5 μm , smooth, dacryoid, phaseoliform to ellipsoid, amyloid. Cheilocystidia 30–80 $\times$ 9–17 μm , lageniform, fusiform, apically gradually or abruptly narrowed into a longer or shorter neck. Some cheilocystidia have granular refringent secretions on the neck. Pleurocystidia similar to cheilocystidia. Caulocystidia with long cylindrical excrescences, 35–120 $\times$ 4–7 μm . Pileipellis hyphae 2–4 μm wide, smooth, rarely with lateral cylindrical excrescences. Clamp connections very rare, present at the hyphae of stipe trama.

HABITAT – On fallen and often buried cones of *Pinus nigra* and *Picea abies*, solitary or in small groups (2–5 basidiomata), spring (April).

SPECIMENS EXAMINED: ROMANIA, IASI COUNTY: Breazu Village, 47°12'45"N 27°31'16"E, 111 m alt., on cones of *P. nigra*, 17.IV.2011, Chinan (I 137185); 2.IV.2012, Chinan (I 137329; GenBank JX297426); near Hlincea Village, 47°07'04"N 27°34'56"E, 104 m alt., on cones of *P. nigra*, 1.IV.2012, Chinan (I 137328; GenBank JX297425); Tomești Village, 47°07'47"N 27°42'14"E, 55 m alt., on cones of *P. nigra*, 16.IV.2011, Chinan (I 137184); 1.IV.2012, Chinan (I 137327; GenBank JX297424); NEAMȚ COUNTY, near Leghin Village, 47°14'12"N 26°13'58"E, 508 m alt., on cones of *P. abies*, 5.IV.2012, Chinan & Fusu (I 137330; GenBank JX297427).

MOLECULAR ANALYSIS – ITS sequences of three Romanian *M. plumipes* specimens were identical (I 137327 and I 137328 from *P. nigra*, and I 137330 from *P. abies*), and the fourth specimen (I 137329 from *P. nigra*) differed by only three transitions (= 0.0038 divergence). The only publicly available sequence of *M. plumipes* (Gene Bank JF908440.1, as *M. strobilicola*) differs in only one nucleotide indel from the sequences of Romanian specimens I 137327, I 137328, and I 137330. In the ML tree, all these sequences are recovered as a highly supported clade, with a bootstrap value of 99 (FIG. 1).

Discussion

Mycena plumipes is widely distributed in Europe (Breitenbach & Kränzlin 1991, Derbsch & Schmitt 1987, Moreau 2003, Robich 2003, Gulden 1966, Gerhold 1989), but it is also mentioned in the Altai Mountains (Singer 1938) and Asiatic Turkey (Solak et al. 1999). It is easy to identify in the field due to its habitat on cones, its nitrous smell, and the whitish fibrils on the base of the stipe.

The Romanian specimens collected from cones of *P. nigra* and *P. abies* morphologically agree with the description provided by Moreau (2003).

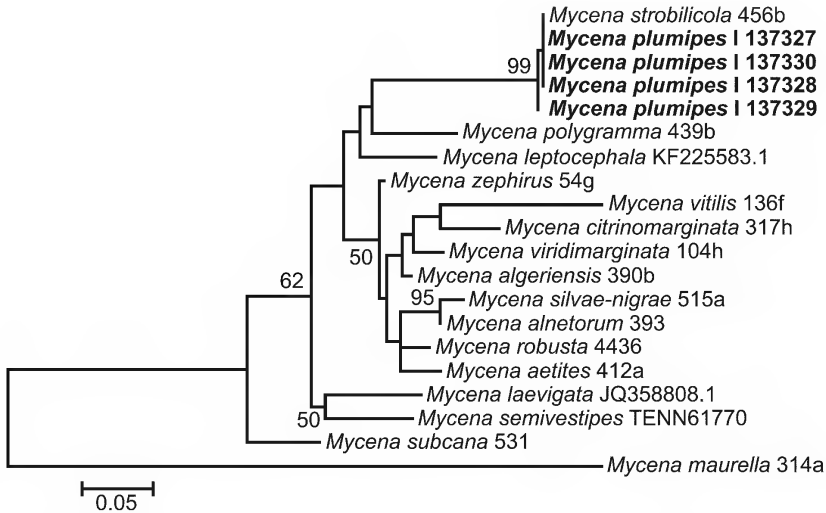


FIG. 1. ML tree based on ITS sequences. Bootstrap values >50, based on 1000 replications, are shown near nodes. Sequences are labelled with voucher number or GenBank accession number. Newly generated sequences are presented in bold font.

In comparing our observations with published data, an important aspect refers to the striation of the pileus, as only some of the specimens from Romania are finely translucently striate at the edge. Moreau (2003) and Roux (2006) describe the edge of the pileus as slightly striate, whereas Kühner (1938), Maas Geesteranus (1988), Breitenbach & Kränzlin (1991), and Robich (2003) state that the pileus is not striate.

The differences between the ITS sequences of *M. plumipes* from Romania are well below the mean intraspecific sequence divergence of 0.025 reported for fungi (Schoch et al. 2012). This confirms the conspecificity of *Mycena* specimens from the cones of *P. abies* and *P. nigra*. The morphologically based identification of the species is further confirmed by the high similarity of our sequences with a sequence available in Gene Bank (accession number JF908440.1) and derived from a specimen collected from Italy, identified as *M. strobilicola* (= *M. plumipes*), in the herbarium of the Museum of Natural History in Venice (Osmundson et al. 2013).

In terms of habitat, in Europe *M. plumipes* is described by most authors as growing on *Picea* cones (Breitenbach & Kränzlin 1991; Derbsch & Schmitt 1987; Emmett et al. 2008; Gulden 1966; Kühner 1938; Maas Geesteranus 1988;

Moreau 2003; Robich 2003, 2006; Roux 2006). However, in some regional species lists it (as *M. strobilicola*) has been reported on pine cones. Gerhold (1989, 1991, 1993, 1994) mentions its presence on pine and spruce cones in Austria, without specifying the species of pine. The species is further reported on pine cones in Asiatic Turkey (Anatolia), specifically on the cones of *Pinus brutia* Ten. (Gezer et al. 2007), *P. nigra* (Gezer et al. 2007, Türkoğlu 2008, Türkoğlu et al. 2007), and *P. pinea* L. (Solak et al. 1999), but without mentioning the diagnosis of this species or highlighting its presence on pine cones. Our results based on molecular analysis and morphology confirm the presence of *M. plumipes* on pine cones as previously reported from Austria and Turkey, an aspect that in the past has been poorly documented and overlooked.

In Romania we found *M. plumipes* in conifer plantations on fallen and buried cones of *Picea abies* and *Pinus nigra*, along with other cone-inhabiting vernal species: *Strobilurus tenacellus* (Pers.) Singer, *S. stephanocystis* (Kühner & Romagn. ex Hora) Singer (on black pine cones), and *S. esculentus* (on Norway spruce cones). From the published data it appears that elsewhere, *M. plumipes* has been found in native forests. Since *Pinus* species are widely distributed, it is intriguing why to date this fungus has been reported on pine cones only from Austria and Turkey.

We found *M. plumipes* in April, specifically in depressions in the soil where the cones gather and the water from melting snow stagnates. The Romanian collections confirm the vernal phenology of *M. plumipes* (Gulden 1966, Kühner 1938, Moreau 2003, Türkoğlu et al. 2007). However, Einhellinger (1977) found this species in Germany in early July. In Asiatic Turkey it seems to have a particular phenology; beside springtime, it has been reported in October (Gezer et al. 2007), November (Gezer et al. 2007, Solak et al. 1999), and December (Türkoğlu 2008, Solak et al. 1999).

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Passalora caesalpinicola* sp. nov. from India on *Caesalpinia bonduc

SHAMBHU KUMAR^{*1} & RAGHVENDRA SINGH²

¹Herbarium Division, Birbal Sahni Institute of Palaeobotany,
53, University Road, Lucknow, U.P., India 226007

²Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University,
Varanasi, U.P., India 221005

*CORRESPONDENCE TO: skumartaxon@gmail.com

ABSTRACT — *Passalora caesalpinicola* on *Caesalpinia bonduc* (Leguminosae, Caesalpinioideae) is described and illustrated as a new species. It differs in several characters from the only other *Passalora* sp. recorded on *Caesalpinia* and can be differentiated from morphologically similar *Passalora* spp. on caesalpinoid hosts.

KEY WORDS — cercosporoid fungi, mycodiversity, morphotaxonomy, foliicolous hyphomycetes

Introduction

Passalora Fr. belongs in the complex of cercosporoid fungi (*Capnodiales*, *Mycosphaerellaceae*). In its current circumscription, this genus is characterized by having pigmented conidiophores, thickened and darkened conidiogenous loci, either consistently internal or internal and external mycelium in vivo, and solitary to catenate, pigmented conidia with thickened and darkened hila (Braun et al. 2013, 2014, 2015; Crous & Braun 2003; Kamal 2010). During the last decade a large number of fungal species from all over the world have been recombined in *Passalora* (Braun et al. 2013, 2014, 2015; Crous & Braun 2003), particularly from India (Kamal 2010). Recently, additional *Passalora* species have been described from India (Singh et al. 2012, 2013; Kumar & Singh 2015), suggesting that the diversity of such fungi is still insufficiently known in this region. A leaf-spotting cercosporoid hyphomycete corresponding to the

current concept of *Passalora* has recently been collected on *Caesalpinia bonduc* in the Nichlaul forest of Uttar Pradesh, India. As it clearly differs from the only *Passalora* species previously described on *Caesalpinia* (also from India) and other morphologically similar *Passalora* species on hosts in *Caesalpinioideae*, we therefore propose here a new species, *Passalora caesalpinicola*.

Materials & methods

Living leaves with fungal symptoms were collected from a subtropical forest (Terai, Nichlaul) in Uttar Pradesh, India. The collections were carried to the laboratory and processed by standard techniques set forth in Hawksworth (1974) and Savile (1962). After the sun-dried and pressed leaves were placed in air tight polyethylene bags, they were kept in paper envelopes with collection notes. Specimens were sectioned by hand or scraped for microscopic observation. Sections from infected leaf areas were mounted in clear lacto-phenol cotton blue mount for observation under an Olympus BX-51 light microscope. Detailed morphological characters were observed (and camera-lucida line drawings made) at 400× and 1000× magnifications, and 30 conidia, hila, and conidiophores and 20 stromata (extremes shown in parentheses) were measured. Identification was made with reference to the current literature and available resident expertise. The holotype is deposited in Herbarium Cryptogamiae Indiae Orientalis, Indian Agricultural Research Institute, New Delhi, India (HCIO) and an isotype was retained in Mycological Herbarium of Birbal Sahni Institute of Palaeobotany, Lucknow, India (BSIPMH). Description and nomenclatural details have been deposited in MycoBank (www.Mycobank.org) and Faces of Fungi (www.facesoffungi.org). Classification of the new taxon is based on current concepts (Braun et al. 2013, 2014, 2015; Cannon & Kirk 2007; Crous & Braun 2003; Farr & Rossman 2015; Kirk et al. 2008; Seifert et al. 2011).

Taxonomy

***Passalora caesalpinicola* Sh. Kumar & Raghv. Singh, sp. nov.**

FIGS 1, 2

MYCOBANK MB 812867

Differs from *Passalora caesalpiniae* by its longer and wider conidiophores and its longer, wider, smooth, and solitary conidia.

TYPE: India, Uttar Pradesh, Nichlaul forest, Mahrajanj, on living leaves of *Caesalpinia bonduc* (L.) Roxb. (*Leguminosae*, *Caesalpinioideae*), 13 February 2007, coll. Shambhu Kumar (Holotype, HCIO 48665; isotype, BSIPMH 30).

ETYMOLOGY: the species epithet is derived from the name of the host genus.

Infection spots hypogenous, circular to irregular, dark brown to black, 1–4 mm diam. COLONIES hypophyllous, effuse, brown. Mycelium internal. Sexual morph: not developed. Asexual morph: Stromata well-developed, sub-epidermal, pseudoparenchymatous, olivaceous-brown, 28–44 µm diam. CONIDIOPHORES macronematous, fasciculate (up to 6), arising from stromata, erumpent, simple, erect to procumbent, divergent, straight, smooth, thick-



FIGURE 1. *Passalora caesalpiniiicola* on *Caesalpinia bonduc*: a. Host plant. b. Infection spots on leaf. c. Infection spots on leaflet. Scale bars = 20 mm.

walled, $\leq 3\times$ transversely euseptate, olivaceous to dark brown, $80\text{--}135 \times 5\text{--}7 \mu\text{m}$. CONIDIOGENOUS CELLS integrated, terminal, polyblastic, conidiogenous loci (scars) thickened and darkened, $2\text{--}3 \mu\text{m}$ wide. CONIDIA solitary, simple, dry, acropleurogenous, smooth, thin-walled, 3–5 times transversely septate, straight to curved, obclavate to obclavate-cylindrical, base rounded, apex obtuse to acute, olivaceous to olivaceous-brown, $25\text{--}65 \times 5\text{--}10 \mu\text{m}$, hilum thickened and darkened, $2\text{--}3 \mu\text{m}$ wide.

COMMENTS— The only *Passalora* species previously described from *Caesalpinia* is *P. caesalpiniae* (Bhalla et al.) U. Braun et al. (Hernández-Gutiérrez & Dianese 2009; Bhalla et al. 2001, as *Phaeoramularia caesalpiniae* from *C. bonducella* [= *C. bonduc*] in India). *Passalora caesalpiniae* differs from *P. caesalpiniiicola* by its amphigenous leaf spots, smaller stromata ($8.5\text{--}13 \times 4.5\text{--}20.5 \mu\text{m}$), shorter narrower verruculose conidiophores ($8\text{--}47 \times 2.5\text{--}5 \mu\text{m}$) arranged in larger fascicules, and its shorter narrower catenate conidia ($4\text{--}30.5 \times 2.5\text{--}6 \mu\text{m}$; Bhalla et al. 2001).

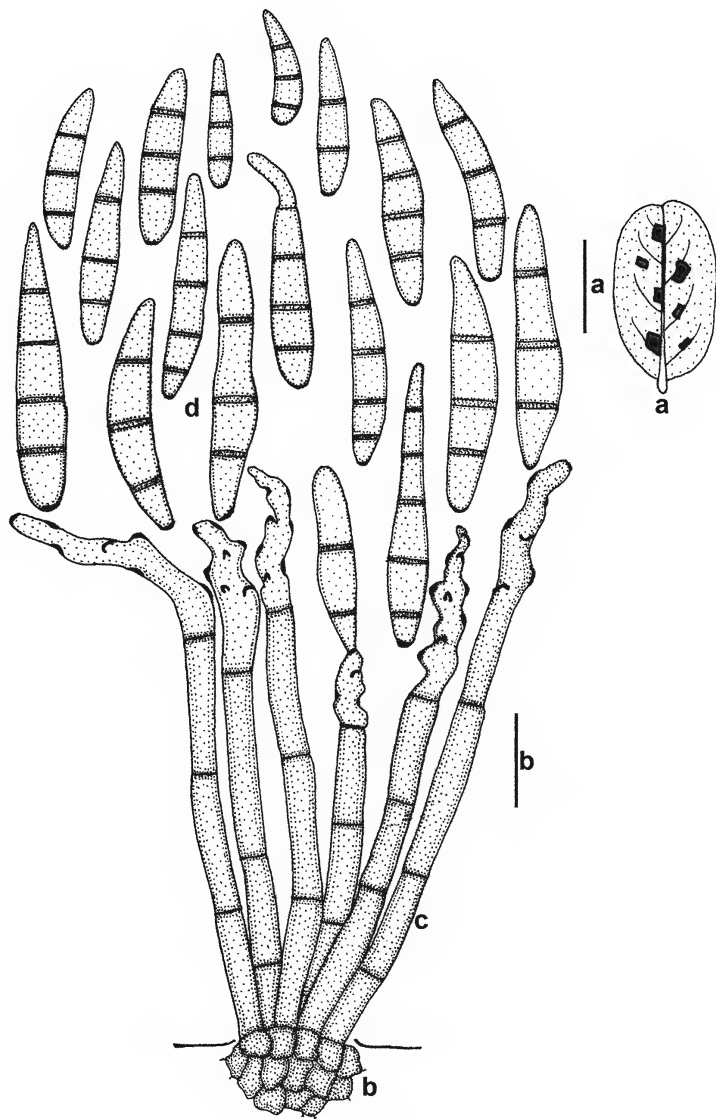


FIGURE 2. *Passalora caesalpiniicola* (holotype, HCIO 48665): a. Infection spots. b. Stromata. c. Conidiophores. d. Conidia. Scale bars: a = 20 mm, b = 20 μ m.

Five other *Passalora* species with solitary conidia and caesalpinioideous resemble *P. caesalpinicola*: *P. aenea* (Cif.) U. Braun & Crous differs by its shorter conidia ($19\text{--}53 \times 4\text{--}6 \mu\text{m}$, 2–5-septate; Braun & Crous 2003), *P. bauhiniicola* U. Braun by its longer conidia ($35\text{--}70 \times 6\text{--}8 \mu\text{m}$, 3–8-septate; Braun 2001), *P. cercidicola* (Ellis) U. Braun by its longer conidia ($20\text{--}80 \times 4\text{--}8 \mu\text{m}$, 3–5-septate; Braun 1995), *P. schizolobii* M.J. Wingf. & Crous by its longer verruculose conidia with more septa ($40\text{--}90 \times 3\text{--}4 \mu\text{m}$, 1–8-septate; Wingfield et al. 2006), and *P. chamaecristae* (Ellis & Kellerm.) U. Braun by its narrower conidia ($31\text{--}65 \times 4\text{--}7 \mu\text{m}$, 1–5-septate; Braun & Mel'nik 1997).

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First record of *Russula anthracina* and its ectomycorrhiza associated with Himalayan cedar from South Asia

SANA JABEEN^{1*}, ABDUL REHMAN NIAZI² & ABDUL NASIR KHALID²

¹Harvard University Herbaria, Department of Organismic and Evolutionary Biology,
Harvard University, Cambridge, MA 02138, USA

²Department of Botany, University of the Punjab,
Quaid-e-Azam Campus-54590, Lahore, Pakistan

* CORRESPONDENCE TO: ectomycorrhzae@gmail.com

ABSTRACT — *Russula anthracina* and its ectomycorrhiza are identified on the basis of morphological characters and molecular analysis. The species is characterized by brittle and blackening basidiomata with pinkish lamellae. Ectomycorrhizal morphotypes are characterized by monopodial pinnate ramification and the presence of typical russuloid cystidia on outer mantle surface. Molecular phylogenetic analysis clustered the ITS sequences generated in this study with other *R. anthracina* sequences. This is the first report of *R. anthracina* from Pakistan and South Asia.

KEY WORDS — *Cedrus deodara*, *Russula* sect. *Compactae*, Swat

Introduction

The genus *Russula* Pers. (Agaricomycetes, Russulales, Russulaceae) is represented by more than 750 species distributed worldwide (Kirk et al. 2008, Das et al. 2013, Arora & Nguyen 2014, Razaq et al. 2014, Dutta et al. 2015). From Pakistan, 26 species of *Russula* have been reported to date (Ahmad et al. 1997, Niazi et al. 2006, Niazi 2008, Razaq et al. 2014, Jabeen et al. 2015). *Russula* species are important ecologically as ectomycorrhizal symbionts associated with many kinds of forest trees (Richardson 1970; Bills et al. 1986; Villeneuve et al. 1989, 1991; Gardes & Bruns 1996; Buyck et al. 1996). Many species are harvested worldwide for consumption (Fogel 1975, Fogel & Trappe 1978, Hu & Zeng 1992, Guo 1992, Rammeloo & Walley 1993, Buyck 1994).

Russula anthracina is characterized in *R. sect. Compactae*, which consists of blackening species. Taxa in the section are quite easy to identify as a group.

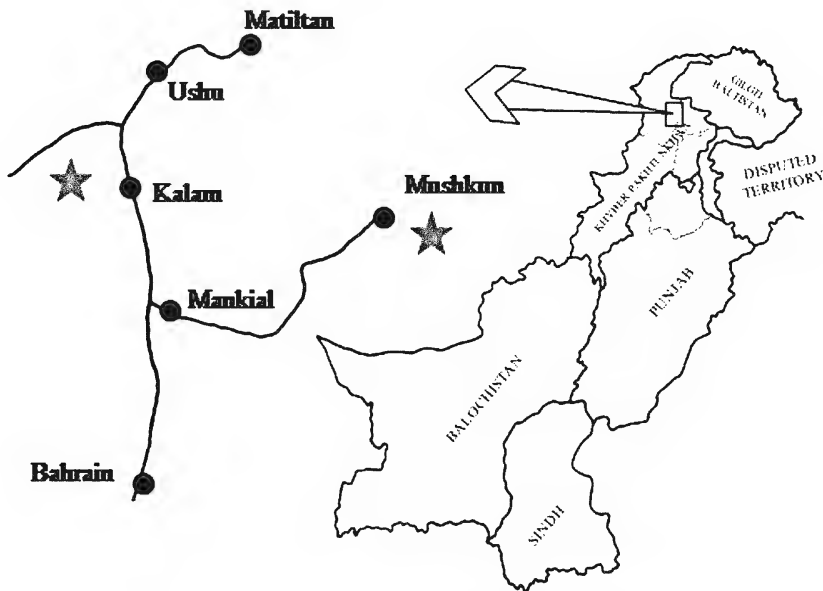


FIG. 1. Map of Pakistan showing sampling sites.

Their squat, robust, and brittle basidiomata are initially white but rapidly become brown to black in all parts (Kibby 2001). However within the section species are less easily distinguished due to their intergrading colors and obscure morphological and anatomical variations. Here, combining morphological and molecular analyses greatly helps in the correct identification of taxa (Arora & Nguyen 2014).

The goal of the current study was to collect ectomycorrhizal fungal fruit bodies and their ectomycorrhizae associated with Himalayan cedar from Pakistan. During the field survey (2012–14) in monsoon season, abundant *Russula anthracina* fruiting bodies were found in coniferous forests of Swat district, Khyber Pakhtunkhwa, Pakistan. The area is surrounded by mountains and has a typical dry temperate climate (Stucki & Khan 1999). The mountains are dominated by forests of *Cedrus deodara* (Roxb. ex D. Don) G. Don along with *Pinus* spp. and *Quercus oblongata* D. Don [= *Q. incana* Roxb., nom. illeg.] (Champion et al. 1965). *Russula anthracina* constituted the most abundant macrofungal taxon observed during 2013 in Kalam (FIG. 1), a lush green area along the river Swat. *Russula anthracina* fruiting bodies and ectomycorrhizae associated with Himalayan cedar were also collected from Mushkun (FIG. 1).

Nuclear ribosomal DNA sequence analyses of the ITS region from fruit bodies and ectomycorrhizal tissues were combined with detailed morphological examination to confirm our Pakistani collections as *R. anthracina*.

Materials & methods

Collection, morphological and anatomical study of basidiomata

Specimens were photographed in the field using a Nikon D70S digital camera. Fresh morphological characters were recorded and colors were designated using Munsell Color System (1975) after which the specimens were preserved by drying over hot air. For detailed anatomical examination, tissues from lamellae, pileipellis and stipitipellis were mounted on glass slides and observed in Phloxine (1%) for better contrast, Melzer's reagent for amyloid basidiospore ornamentation, and KOH (5%) for colored hyphae using a Meiji Techno MX4300H microscope. Anatomical features were measured using a Carl Zeiss Jena ocular micrometer and line drawing were made using a Leitz Wetzlar camera lucida.

The abbreviation 'n/b/p' indicates 'n' basidiospores measured from 'b' basidiomata from 'p' collections. Basidiospore dimensions are given as length $\times$ width ($l \times w$), with extreme values given in parenthesis. $Q = l/w$ ratio of individual spores; avQ = average Q of all spores $\pm$ standard deviation. Collector's numbers prefixed with K refer to Kalam and SJ (basidiomata) and SA (ectomycorrhizae) refer to sequence codes. Voucher specimens were deposited in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Collection, isolation, morphological and anatomical study of ectomycorrhiza

15-cm³ soil blocks were dug a few centimetres away from tree trunks using extreme care to insure that the fine roots in the soil block belonged to the selected tree species. Twenty soil cores were taken from each sampling site. In laboratory, each soil core was soaked in water for few hours to loosen the adhering soil particles and then put on a 2 mm sieve under running water to separate the roots from the soil. The ectomycorrhizae (EcM) were carefully sorted out into morphotypes under incandescent light and then under a Meiji Techno EMZ-5TR stereomicroscope. The morphotypes were cleaned under the stereomicroscope using a fine brush. Morphologically identical morphotypes were kept in McCartney bottles in distilled water for later examination. Replicates of these morphotypes were kept at 8 °C in eppendorf tubes containing 1 mL of 2% CTAB buffer for molecular analysis. Morphological characters were noted under the stereomicroscope. For anatomical details, mantle layers (inner and outer) and radiating hyphae were mounted in trypan blue stain (Agerer 1991). Hyphae were measured using an ocular micrometer and drawn using a camera lucida.

DNA extraction, amplification and sequencing

For genomic DNA extraction, 2 mg of fungal tissue was taken from the gills in an eppendorf tube, 2–4 ectomycorrhizal root tips were taken in a separate eppendorf tube. DNA was extracted following modified CTAB method (Bruns 1995). Using forward primer ITS1F (5'-CTGGTCATTTAGAGGAAGT-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990), internal transcribed spacer (ITS)

regions along with the central 5.8S region of nuclear ribosomal DNA (nrDNA) were amplified following Gardes & Bruns (1993). The PCR products were sent to Macrogen Inc. (Korea) for sequencing.

Molecular phylogenetic analysis

Consensus sequences were generated from the sequences obtained by both primers in BioEdit software. These sequences were then BLAST searched at NCBI (<http://www.ncbi.nlm.nih.gov/>). Sequences with closest match were selected from GenBank to reconstruct phylogeny (sequences with less query cover and negative E values were not included). Published sequences of the most closely related species were also included in the final data set. *Boletus reticuloceps* (M. Zang et al.) Q.B. Wang & Y.J. Yao was chosen as outgroup (Shimono et al. 2004). Multiple sequences were aligned using online MUSCLE by EMBL-EBI (<http://www.ebi.ac.uk/Tools/msa/muscle/>). The sequences were trimmed with the conserved motifs 5'-(...GAT)CATTAA... and ...GACCT(CAAA...)-3' and the alignment portions between them were used for phylogenetic analysis. Maximum likelihood (ML) analysis was performed using General Time Reversible model (Nei & Kumar 2000) in MEGA6 software (Tamura et al. 2013) to test the phylogeny at 1000 bootstraps. Percentage identity and divergence in nrDNA-ITS of the taxa were analysed using MegAlign (DNASar, Inc.). Sequences generated in this study were submitted to GenBank.

Russula anthracina Romagn., Bull. Mens. Soc. Linn. Lyon 31: 173 (1962) Figs 2, 3

BASIDIOMATA medium to large. PILEUS 4.5–8.6 cm broad, depressed from the middle; black to greyish black (10YR1/2) to grey (10YR2/2), dark brown (10YR2/4) to light brown (10R3/6) and pinkish (5YR9/2) in the form of random patches; surface dry, smooth, reflective; margins smooth, incurved when young becoming straight at maturity. LAMELLAE ≤ 0.3 cm broad, pinkish (5YR9/2), regular, adnexed, subdistant to close, edge entire. LAMELLULAE frequent, single-tiered, variable in length. STIPE 3.3–5.1 $\times$ 1.1–2.5 cm, white, central, cylindrical, slightly narrower towards the apex. Surface smooth to finely wrinkled.

BASIDIOSPORES [n/b/p = 40/4/2] (5.4–)7.3–8.0(–8.3) $\times$ (4.7–)6.1–7.0(–7.8) μm , Q = 1.03–1.23, avQ = 1.12 $\pm$ 0.04; globose to subglobose, echinulate, echines ≤ 0.2 μm ; apiculus prominent; guttulate, pale yellow in 5% KOH, amyloid in Melzer's reagent. BASIDIA 40.6–66 $\times$ 9.7 – 10.8 μm , clavate, wall ≤ 3 μm , pale yellow in 5% KOH, guttulate. PLEUROCYSTIDIA 68.4–110 $\times$ 5.2–5.9 μm , fusoid, guttulate, pale yellow in 5 % KOH. CHEILOCYSTIDIA 52.7–71 $\times$ 5.4–6.4 μm , fusiform, guttulate, pale yellow in 5% KOH. PILEIPELLIS hyphae 3.8–5.1 μm wide, filamentous, branched, pale yellow to transparent in 5% KOH, septa infrequent, clamp connections and pileocystidia not observed. STIPITPELLIS hyphae 3.1–4.7 μm diam., filamentous, branched, hyaline to pale yellow, rarely septate, clamp connections not observed.



FIG. 2. *Russula anthracina* basidiomata. A. LAH35009; B. LAH35008. Scale bars = 0.5 cm.

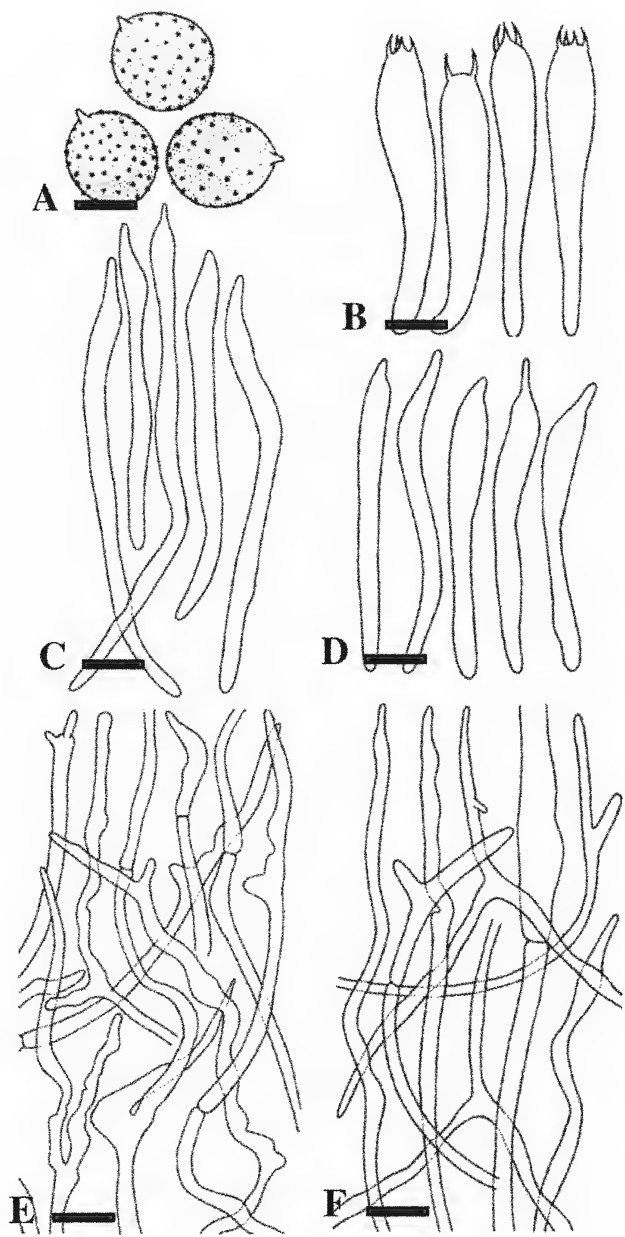


FIG. 3. *Russula anthracina* (LAH35008). A. basidiospores; B. basidia; C. pleurocystidia; D. cheilocystidia; E. hyphae from stipitipellis; F. hyphae from pileipellis. Scale bars: A = 4.5 μm ; B, F = 12 μm ; C = 20.5 μm ; D = 10 μm ; E = 14 μm .

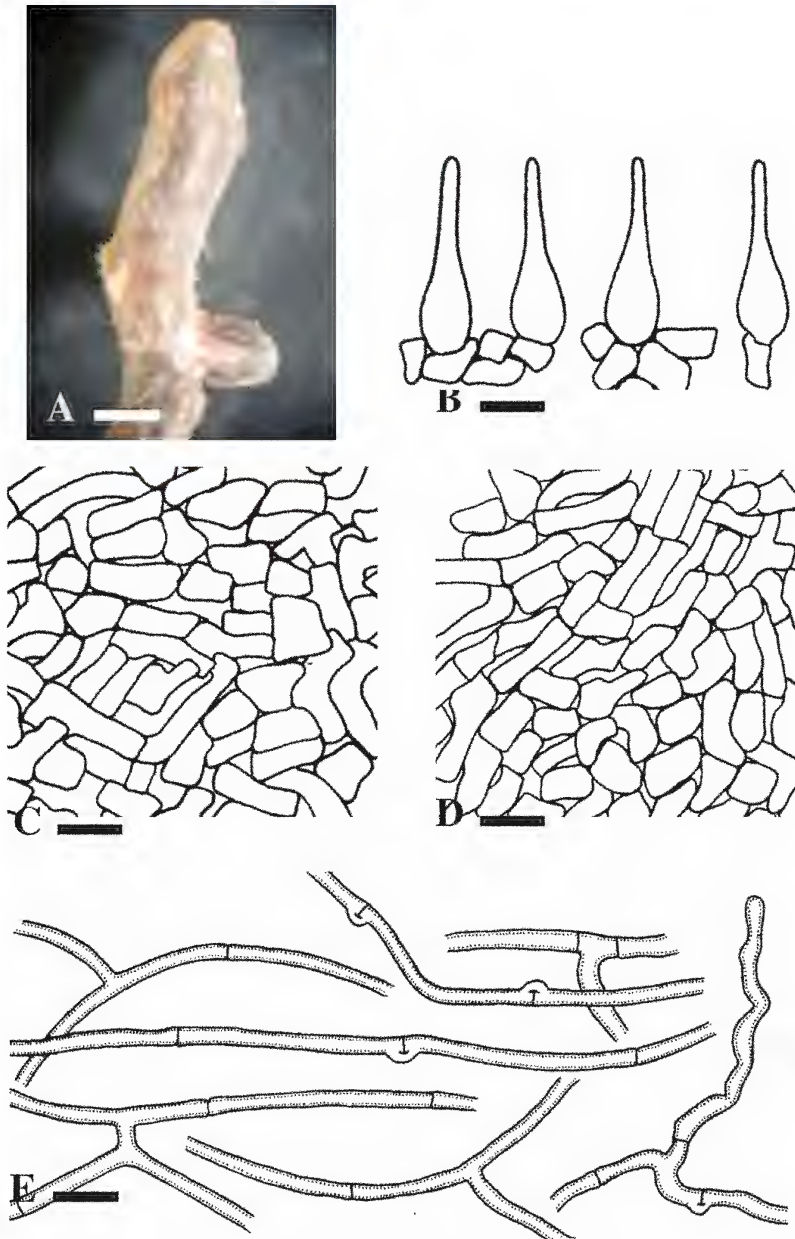


FIG. 4. *Russula anthracina* ectomycorrhiza (LAH-EM2-2013). A. ectomycorrhizal system; B. cystidia; C. inner mantle layer; D. outer mantle layer; E. emanating hyphae. Scale bars: A = 0.7 mm; B = 8.5 μ m; C = 12 μ m; D = 14.5 μ m; E = 25 μ m.

MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA PROVINCE, SWAT DISTRICT, Kalam, 2070 m asl, on soil under *Cedrus deodara*, 3 Sep 2013, Sana Jabeen K3; SJ5 (LAH35008; GenBank KR011879); Mushkun, 2500 m asl, under *Cedrus deodara*, 5 Sep 2013, Sana Jabeen K4-22; SJ19 (LAH35009; GenBank KR011880).

Morphological characterization of EcM

FIG. 4

ECTOMYCORRHIZAL SYSTEM monopodial pinnate, 0.7–1.2 cm long, axis ≤ 1 mm diam., unramified ends straight, cylindrical, greyish brown (10YR7/4) to dark brown at maturity, tip greyish brown (10YR7/4). MANTLE greyish brown to transparent, distinct, smooth surface, lustre matte, cortical cells visible under the mantle surface at some tips. RHIZOMORPHS not observed. EMANATING HYPHAE transparent, rare, sometimes frequent at few root tips, straight, cylindrical rarely branched. CYSTIDIA frequent.

OUTER MANTLE LAYER cells $14.6 \times 8.1 \mu\text{m}$, INNER MANTLE LAYER cells $12.9 \times 6.1 \mu\text{m}$. Cells in both layers pseudoparenchymatous, yellowish to transparent, angular to somewhat roundish or elongated (mantle type L; Agerer 1987–2002), contents clear.

EMANATING HYPHAE elongated, transparent, straight, cylindrical, $\leq 3.9 \mu\text{m}$ in diameter, wall $\leq 0.7 \mu\text{m}$ thick, surface smooth, septate, septa frequent, 2.0–49.3 μm apart, clamp connections rare, ornamentation absent, contents clear, ramification rather frequent, Y-shaped, anastomoses H-shaped, frequent, without septa and clamp connections. CYSTIDIA lageniform, frequent, 23.9 μm long, strongly inflated base, $\leq 6.8 \mu\text{m}$ diam., attachment point $\leq 5 \mu\text{m}$ wide, neck straight, 4.9 μm diam. with 3.7 μm tip.

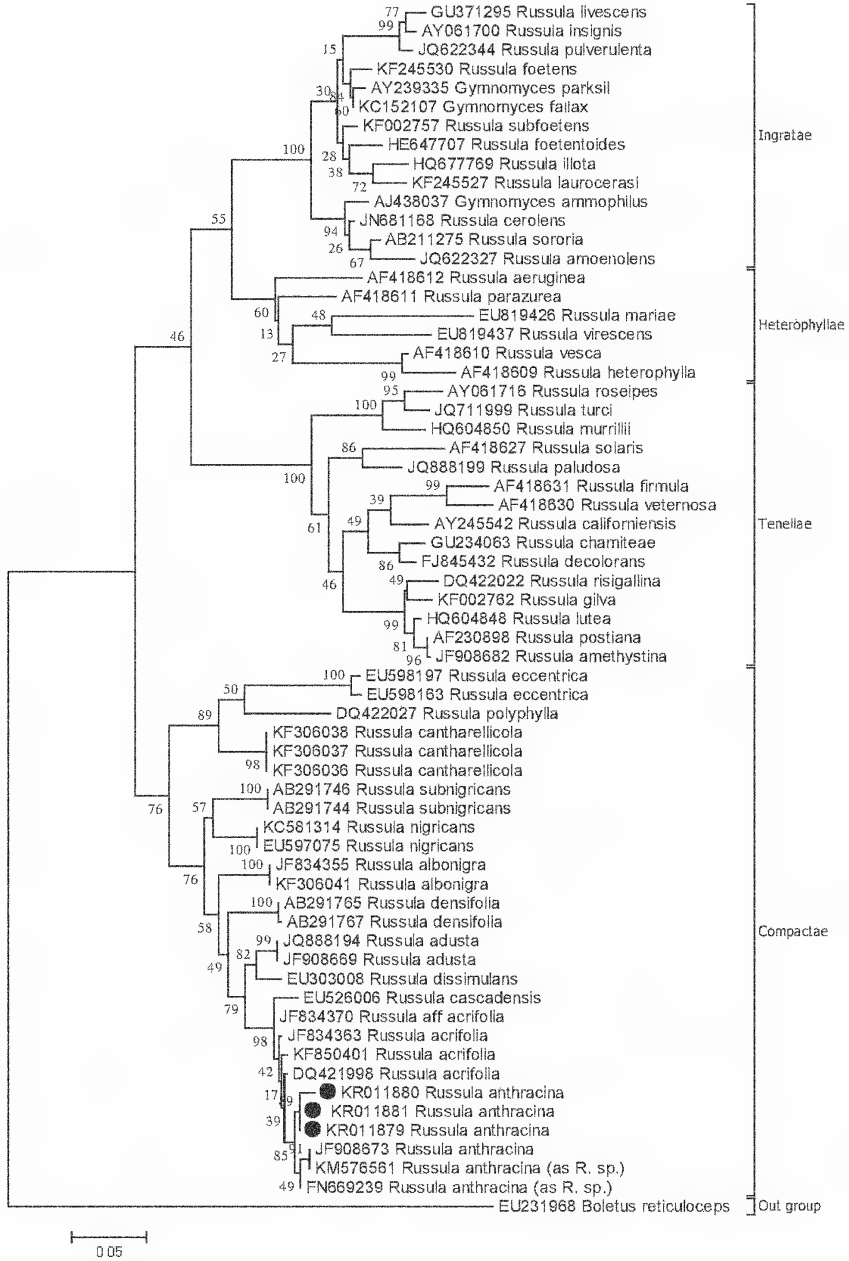
MATERIAL EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA PROVINCE, SWAT DISTRICT, Mushkun, 2500 m asl, associated with *Cedrus deodara*, 5 Sep 2013, Sana Jabeen K4-5; SA99 (LAH-EM2-2013; GenBank KR011881).

Molecular phylogenetic characterization

FIG. 5

Sequencing of the *Russula anthracina* PCR products using ITS1F/ITS4 yielded 697–752 base pairs. A 598-base pair consensus sequence was obtained by trimming the motifs. BLAST of *R. anthracina* (KR011881) revealed it as 99% identical with KF679819, FN669239, and KM576561 (100% query cover, 0.0 E value) and a 99% identity with JF908673 (93% query cover,

FIG. 5. Molecular phylogenetic analysis of *Russula* spp. inferred by the Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 64 nucleotide sequences. New sequences generated from Pakistan are marked with ●. There were a total of 828 positions in the final dataset.



0.0 E value). Published sequences from different *Russula* sections were included to reconstruct a phylogeny with *Boletus reticulocephus* (EU231968) as outgroup. The analysis revealed four major clades (/compactae, /heterophyllae, /ingratae, /tenellae) corresponding with the taxonomic sections (FIG. 5). Species within these clades were clustered with strong bootstrap, supported by morphological characters. Within /compactae, *R. acrifolia* Romagn., *R. adusta* (Pers.) Fr., *R. albonigra* (Krombh.) Fr., *R. anthracina*, *R. cantharellicola* Arora & N.H. Nguyen, *R. cascadiensis* Shaffer, *R. densifolia* Secr. ex Gillet, *R. dissimulans* Shaffer, *R. eccentrica* Peck, *R. nigricans* Fr., *R. polyphylla* Peck, and *R. subnigricans* Hongo clustered with 76% bootstrap support.

Discussion

Russula anthracina can be distinguished from *R. acrifolia* by its pinkish tinged lamellae (Romagnesi 1967, Galli 1996, Sarnari 1998), which are pale cream in *R. acrifolia*. Anatomically, in *R. anthracina* the pileus cuticle lacks pileocystidia, which are numerous with frequently diverticulate tips in *R. acrifolia*. *Russula albonigra* is distinguished from *R. anthracina* and *R. acrifolia* by its rapidly blackening flesh and rather large, unforked pileocystidia (Rayner 1985, Kibby 2001).

Russula anthracina is an ectomycorrhizal symbiont of both deciduous and coniferous trees (Gminder et al. 2000, Bahram et al. 2011, Suz et al. 2014). We describe and illustrate this ectomycorrhiza here for the first time in detail. Morphologically it differs from the ectomycorrhiza of the closely related *R. acrifolia* (Agerer 1996) by its monopodial pinnate branching pattern and greyish brown to transparent mantle surface with cellular hyphae and typical russuloid cystidia without an apical knob.

In phylogenetic analysis, our Pakistani sequences showed a 0.2–1% genetic divergence from *R. acrifolia* and clustered with European *R. anthracina* sequences from Austria, Estonia, and Italy with 85% bootstrap support. *Russula anthracina* is a European species (Romagnesi 1967, Petersen & Vesterholt 1993, Einhellinger 1994, Hallingbäck & Aronsson 1998, Gminder et al. 2000, Piltaver et al. 2002, Legon & Henrici 2005, Kränzlin 2005, Adamčík et al. 2006, Holec & Beran 2006, Dimou et al. 2008, Ronikier & Adamčík 2009, Bahram et al. 2011, Cabo 2012, Kasom & Karadelev 2012, Osmundson et al. 2013, Suz et al., 2014), but it has also been recorded in America (Roberts 2007, Kuo 2009) and Africa (El Kholfy et al. 2011, Kinge et al. 2013). From western Asia, it is reported from Turkey (Yalç et al., 2006, Günç Ergönül et al. 2013, Sesli & Denchev 2014). Our *Russula anthracina* collections represent an addition to the mycobiota of Pakistan and a first report of the species from South Asia.

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***Sympodiosynnema*, a new genus of dematiaceous hyphomycetes from southern China**

Ji-WEN XIA, YING-RUI MA, JIAN-MEI GAO, ZHUANG LI* & XIU-GUO ZHANG*

Department of Plant Pathology, Shandong Agricultural University, Taian, 271018, China

*CORRESPONDENCE TO: sdau613@163.com, liz552@126.com

ABSTRACT — *Sympodiosynnema* gen. nov. is illustrated and described from dead stems of an unidentified plant in Hainan, southern China. The genus is characterized by solitary dry naviculiform conidia, sympodially proliferating conidiogenous cells, and synnematus conidiophores.

KEY WORDS — *Sympodioplanus*, conidial fungi, taxonomy

Introduction

During a survey of dematiaceous hyphomycetes colonizing diverse plant habitats from the forests of Hainan, China, a fungus that does not match any existing genera was collected growing on unidentified dead twigs. A review of the literature and morphological studies revealed that it represents an undescribed genus (Ellis 1971, 1976, Subramanian 1971, Matsushima 1975, 1983, 1985, 1989, 1993, 1995, Carmichael et al. 1980, Castañeda-Ruiz 1986, Castañeda-Ruiz & Kendrick 1990a,b, 1991, Wu & Zhuang 2005, Seifert et al. 2011).

The new genus, *Sympodiosynnema*, is described and illustrated. It is characterized by synnematus conidiophores and polyblastic and sympodially proliferating conidiogenous cells that produce solitary naviculiform dry conidia. Specimens are deposited in the Herbarium of Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

***Sympodiosynnema* J.W. Xia & X.G. Zhang, gen. nov.**

MYCOBANK MB 815649

Differs from *Sympodioplanus* by possessing synnematus conidiophores.

TYPE SPECIES: *Sympodiosynnema elegans* J.W. Xia & X.G. Zhang

ETYMOLOGY: *Sympodio-* referring to the similar genus *Sympodioplanus*; *synnema*, referring to the synnematus conidiophores.

ASEXUAL FUNGUS. COLONIES on the natural substrate effuse, brown. Mycelium mostly immersed, composed of branched, septate, hyaline to brown, smooth hyphae. CONIDIOMATA synnematal unbranched, erect, with brown stalks, consisting of compact aggregation of parallel conidiophores. CONIDIOPHORES macronematous, erect, unbranched, septate, pale brown to brown, divergent towards the distal part of the synnema. CONIDIOGENOUS CELLS polyblastic, integrated, terminal, sympodial, hyaline to pale brown. Conidial secession schizolytic. CONIDIA solitary, dry, naviculiform, euseptate, pale brown.

***Sympodiosynnema elegans* J.W. Xia & X.G. Zhang, sp. nov.**

FIG. 1

MYCOBANK MB 815650

Differs from *Sympodioplanus capensis* by its synnematus conidiophores with sympodial proliferations and its larger conidia and from *S. goaensis* by its synnematus conidiophores with sympodial proliferation and its more septate conidia.

TYPE: China, Hainan Province: Jianfengling, on dead stems of unidentified broadleaf tree, 22 Apr. 2014, J.W. Xia (Holotype, HSAUP H6447; isotype, HMAS 245682).

ETYMOLOGY: *elegans*, referring to the elegant conidia.

COLONIES on the natural substrate effuse, brown. Mycelium mostly immersed, composed of branched, septate, hyaline to brown, smooth, 1.5–3 µm wide hyphae. CONIDIOMATA synnematal, unbranched, erect, with brown stalks, consisting of a compact aggregation of parallel conidiophores, terminating in brown fertile heads, ≤300 µm long, 25–35 µm wide in the middle with a base ≤45 µm wide and an apex 110 µm wide. CONIDIOPHORES macronematous, erect, unbranched, septate, pale brown to brown, 3–5 µm wide, divergent towards the distal synnema. CONIDIOGENOUS CELLS polyblastic, integrated, terminal, sympodial, thin-walled, smooth, hyaline to pale brown, bearing flat scars, sometimes slightly obscured. Conidial secession schizolytic. CONIDIA solitary, dry, naviculiform, 3–5-euseptate, smooth, pale brown, 22.5–28 × 6–8 µm.

COMMENTS – *Sympodiosynnema* demonstrates unique morphological and ontogenetic features. Its macronematous conidiophores form distinct synnemata with a sympodial proliferation of the conidiogenous cells and produce solitary dry naviculiform conidia. Conidial fungi characterized by sympodial proliferation represent a large group, among which only *Sympodioplanus*

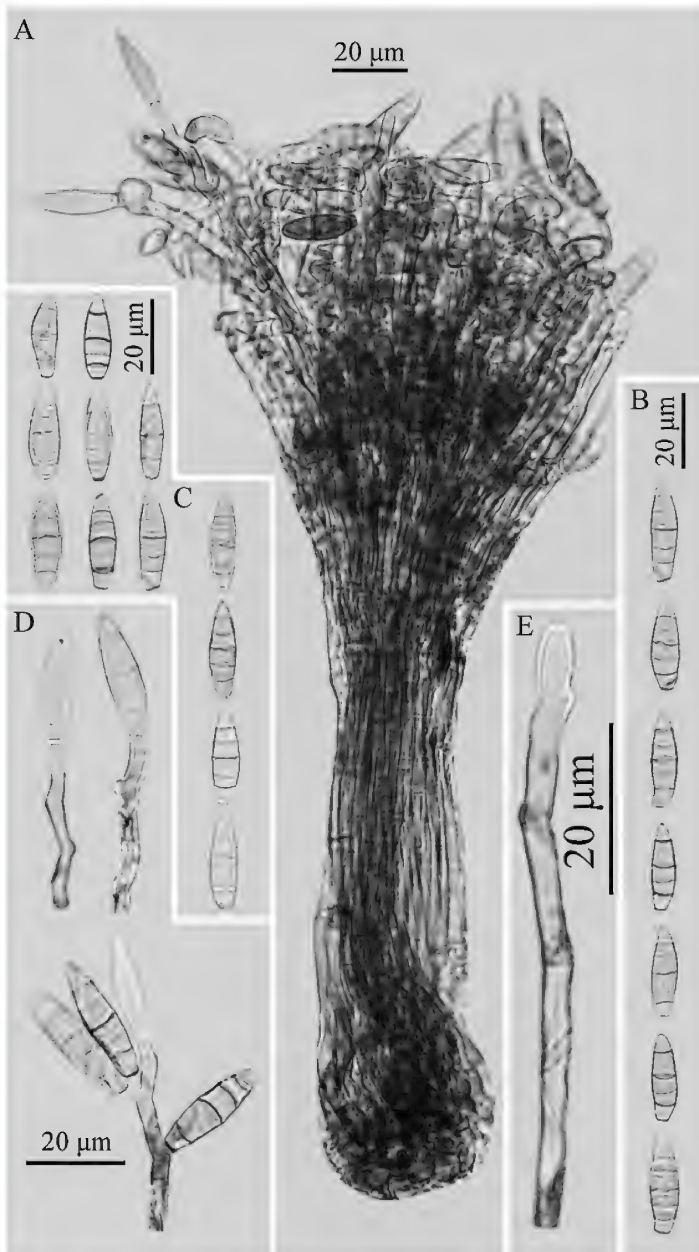


FIG. 1. *Sympodiosynnema elegans* (holotype, HSAUP H6447).

A. synnema; B, C. conidia; D. conidiogenous cells and conidia;

E. conidiogenous cells and conidiogenous loci.

R.C. Sinclair & Boshof (Sinclair et al. 1997) has conidiogenous cells and conidia that are morphologically similar to *Sympodiosynnema*. *Sympodioplanus*, however, is distinguished by mononematous conidiophores.

In conidial shape, *Sympodiosynnema elegans* is most similar to *Sympodioplanus capensis* R.C. Sinclair & Boshof, which differs by its smaller conidia ($13\text{--}16 \times 2.5\text{--}4\text{ }\mu\text{m}$; Sinclair et al. 1997). The also similar *S. goaensis* J. Pratibha can be distinguished by its less septate conidia (1–2-septate; Pratibha 2013).

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New records from Lithuania of fungi alien to Europe

JURGA MOTIEJŪNAITĖ^{1*}, ERNESTAS KUTORGA², JONAS KASPARAVIČIUS¹,
VAIDOTAS LYGIS¹ & GODA NORKUTĖ¹

¹ Institute of Botany of Nature Research Centre,
Žaliųjų Ežerų Str. 49, Vilnius, LT-08406 Lithuania

² Department of Botany and Genetics, Vilnius University,
Saulėtekio Av. 7, Vilnius, LT-10222 Lithuania

* CORRESPONDENCE TO: jurga.motiejunaite@botanika.lt

ABSTRACT — First records from Lithuania of the ascomycete *Ophiostoma novo-ulmi* and basidiomycetes *Clathrus archeri*, *Leucocoprinus cepistipes*, and *Stropharia rugosoannulata* are presented. All four species are alien to Europe and two, *C. archeri* and *S. rugosoannulata*, have never been recorded in the eastern part of the Baltic Sea region before. Also, a reassessment of the status in Lithuania of the alien pathogen *Hymenoscyphus fraxineus* and its close relative, the non-pathogenic *H. albidus* indigenous to Europe, indicates that only *H. fraxineus* occurs in Lithuania. Descriptions of the examined fungi are presented, and remarks on their habitats and distribution are provided.

KEY WORDS — anthropogenic habitats, distribution, invasive species, non-native fungi, saprobes

Introduction

Precise data on diversity and distribution of fungi, their geographical ranges, and habitat requirements are often lacking even for comparatively well-studied regions. Fragmentary baseline knowledge of fungal taxonomy, ecology, and geographical distribution is probably one reason why research on alien organisms and their effect on ecosystems has so far paid insufficient attention to fungi (Pyšek et al. 2008), with the exception of some invasive pathogenic species that cause conspicuous damage to vegetation or wildlife (Desprez-Loustau et al. 2010). Until recently, the spread of alien mycorrhizal and saprobic macromycetes in particular was treated as a mycological curiosity, although

lately the economical and ecological importance of alien non-pathogenic fungi has been acknowledged (Richardson et al. 2000, Desprez-Loustau et al. 2007, Vellinga et al. 2009, Nuñez & Dickie 2014). Nonetheless, the general knowledge of geographical distribution of alien fungi is far from complete (Kreisel 2006, Desprez-Loustau et al. 2010, Santini et al. 2013) as information on new localities of already established alien fungi and on new arrivals continuously accumulates. Moreover, accurate identification is often an issue for reports on the occurrence of alien macromycetes — e.g., *Amanita phalloides* (Fr.) Link in North America; Pringle & Vellinga 2006 — and plant pathogens — e.g., *Hymenoscyphus fraxineus* (Baral & Bemmam 2014, Gross et al. 2014) and subspecies and hybrids of *Ophiostoma novo-ulmi* (Brasier 1991, Brasier & Buck 2001, Brasier & Kirk 2001) — in Europe.

In Lithuania, the first records of fungi (including aliens) appeared almost 200 years ago; since then the number of non-native species records has continuously increased, although so far there is still no comprehensive list of such fungi in the country. Here we report and discuss the first cases of occurrence of four fungal species that are alien in Europe, and revise the status of an invasive ash dieback pathogen, *Hymenoscyphus fraxineus*, and its close relative indigenous saprobe, *H. albidus* (Gillet) W. Phillips, in Lithuania.

Materials & methods

EXAMINATION OF FUNGAL FRUIT-BODIES. Fresh basidiomata of *Leucocoprinus cepistipes* and *Stropharia rugosoannulata* and dried basidiomata of *Clathrus archeri* were studied. Additionally, color photographs of *C. archeri* in situ were examined. Microscopical features were examined under a Nikon Eclipse Ci-S light microscope. Squashed preparations were mounted in water and in 5% KOH solution. All measurements were made in water. Herbarium specimens previously identified as *Hymenoscyphus albidus* were examined for presence of simple septa or croziers at the bases of asci. Squashed sections or fragments of ascomata were mounted in 3% KOH + Lugol's iodine solution or Congo Red and studied under an Olympus BX51 light microscope. All descriptions are based on Lithuanian material.

MOLECULAR IDENTIFICATION OF *OPHIOSTOMA NOVO-ULMI* ISOLATES. A total of 49 *O. novo-ulmi* isolates were recovered from wood of wilting branches of 20 *Ulmus glabra* and 29 *U. minor* trees growing in various parts of Lithuania (for details of the localities see Norkutė 2013). In the laboratory, the fungi were isolated and cultured according to Brasier (1981) and Bakys et al. (2009). All 49 isolates that were morphologically similar to *O. novo-ulmi* based on cultural descriptions by Brasier (1991) and Kirisits & Konrad (2004) were assigned for DNA extraction and grown for two weeks on liquid Hagem media in static cultures at room temperature. Protocols for DNA extraction and PCR amplifications followed Kären et al. (1997), and the ribosomal ITS region was sequenced by Macrogen Inc. (Seoul, Korea) using two primers (ITS1F and ITS4; White et al. 1990). All obtained sequences were identical and identified via a NCBI BLAST

database (www.ncbi.nlm.nih.gov) search as *O. novo-ulmi*. The representative sequence of the Lithuanian *O. novo-ulmi* (LPM ON3; for details see below) has been deposited at GenBank (accession no. KJ677112).

PCR-RFLP of the *cerato-ulmin* (*CU*) and colony type (*COL1*) genes was used to identify *O. novo-ulmi* subspecies and hybrids following the protocol developed by Konrad et al. (2002): DNA of all 49 isolates was amplified using DreamTaq™ Green DNA polymerase (Fermentas, Vilnius, Lithuania) following the manufacturer's instructions with a different annealing temperature for each primer (68°C for *CU*, 58°C for *COL1*). The restriction enzymes *Bfa*I and *Hph*I (Fermentas, Vilnius, Lithuania) were used in the RFLP process according to manufacturer instructions and following recommendations by Konrad et al. (2002). Restriction products were visualized in 2% agarose gel (90 min at 120 V in sodium borate (SB) buffer; FIG. 1). Assignment of the screened *O. novo-ulmi* isolates to subspecies or hybrids followed Konrad et al. (2002).

SPECIMEN ACCESSIBILITY. All specimens cited below are deposited in fungal collections of either the Herbarium of the Institute of Botany of Nature Research Centre, Vilnius, Lithuania (BILAS) or the Herbarium of Vilnius University, Vilnius, Lithuania (WI). Isolates of *O. novo-ulmi* are stored in the culture collection of the Laboratory of Phytopathogenic Microorganisms at the Institute of Botany of Nature Research Centre, Vilnius, Lithuania (LPM).

Nomenclature follows MycoBank Fungal Databases (<http://www.mycobank.org>).

Clathrus archeri (Berk.) Dring, Kew Bull. 35: 29. 1980.

Immature basidiomata globose to ovoid, dirty whitish, growing solitary or gregarious. Mature basidiomata with 4–6 (most commonly five) receptaculum arms initially fused at the tips, later splitting and spreading outwards; arms tapered to apices, bright red on the inner (upper) surface and pale red elsewhere, finely reticulately pitted, with dark green gleba. Receptaculum of rounded cells, 40–50 µm diam. Gleba emitting a strong carrion-like odor (persistent even in dried material). Stem with the base encased in a whitish volva. Basidiospores cylindric-ellipsoid, hyaline, greenish in mass, smooth-walled, 4.5–6 × 2–2.5 µm. Basidia not observed.

SPECIMEN EXAMINED: LITHUANIA, KRETINGA DISTR., northern edge of Kartena village, 55°56'N 21°28'E, 1 Sept 2011, E. Slušnyté (BILAS 49286).

HABITAT IN LITHUANIA: On soil on a semi-open slope, covered with shrubs and in herbaceous vegetation between planted young *Picea abies* and *Pinus sylvestris*.

NOTES: Originally described from Tasmania, and indigenous in Australia and New Zealand, *Clathrus archeri* has spread to Europe, North America, and Asia as an alien species. Since it was first recorded from France in 1914, the fungus has spread widely across western and central Europe (Michael et al. 1986, Pegler et al. 1995, Hansen & Knudsen 1997, Birsan et al. 2014). The records of *C. archeri* closest to Lithuania are from Poland, mostly from the southern part where it has a status of a spreading species, although the reports from

northern Poland are few and at the present time confined to the coastal areas with a milder climate (Miądlukowska 1995, Halama et al. 2010, Szczepkowski & Obidziński 2012). In Lithuania, the fungus was found in the west, also characterized by a milder climate influenced by the Baltic Sea. Kreisel (2006) noted that elsewhere in Europe, *C. archeri* continues to spread northwards, where it has already become established in southern Sweden (M. Jeppson, personal communication). Our present record is the first for the eastern Baltic Sea region.

Hymenoscyphus fraxineus (T. Kowalski) Baral, Queloz & Hosoya, IMA Fungus 5: 79. 2014.

Ascomata developing on blackened (stromatized) petioles of fallen previous year's leaves of *Fraxinus excelsior*, gregarious, superficial, stipitate, cup-shaped, 1–4 mm diam. Hymenium shallowly concave or plane, whitish or cream to ochraceous. Margin even or sinuate, hairless. Receptacle concolorous with the hymenium, somewhat striate. Stipe obconical to cylindrical, concolorous with the receptacle, blackened at the base, ≤ 1.2 mm long. Exterior covered by short hyphal outgrowths, especially on stipe. Ectal excipulum a textura prismatica (-porrecta), cells with slightly gelatinized walls. Medullary excipulum a hyaline textura intricata. Asci arising from croziers, cylindric-clavate, 8-spored, $85\text{--}120 \times 8\text{--}14$ μm , apical pore blue in Lugol's solution. Ascospores cylindrical to cylindric-clavate, straight or slightly curved, often inequilateral, usually scutuloid, with internal droplets, aseptate or occasionally 1-septate, $16\text{--}24 \times 3\text{--}5$ μm . Paraphyses cylindrical, apically unthickened or slightly inflated ≤ 3 μm .

SPECIMENS EXAMINED: LITHUANIA, KĖDAINIAI DISTR., Stebuliai forest, $55^{\circ}19'N$ $24^{\circ}07'E$, 21 Sept 1999, E. Kutorga (BILAS 34422), 29 Aug 2000, E. Kutorga (WI 7344); Vilainiai forest, $55^{\circ}18'N$ $24^{\circ}02'E$, 21 Sept 1999, E. Kutorga (BILAS 34426), 30 Aug 2000, E. Kutorga (WI 7345); Pašiliai forest, $55^{\circ}14'N$ $23^{\circ}58'E$, 1 Jun 2000, E. Kutorga (WI 7341); Lančiūnava forest, $55^{\circ}20'N$ $24^{\circ}12'E$, 29 Aug 2000, E. Kutorga (WI 7342); Berankiškis forest, $55^{\circ}14'N$ $24^{\circ}08'E$, 29 Aug 2000, E. Kutorga (WI 7343), 3 Oct 2001, E. Kutorga (BILAS 25151), 2 Oct 2002, E. Kutorga (BILAS 34425); Juodkiškiai forest, $55^{\circ}16'N$ $24^{\circ}02'E$, 30 Aug 2000, E. Kutorga (WI 7346); Šilainėliai forest, $55^{\circ}14'N$ $24^{\circ}02'E$, 3 Oct 2002, E. Kutorga (BILAS 33994); ŠIRVINTOS DISTR., Širvintos forest, $55^{\circ}03'N$ $24^{\circ}58'E$, 10 Sept 1997, E. Kutorga (WI 7409); UKMERGĖ DISTR., Taujėnai forest, $55^{\circ}26'N$ $24^{\circ}39'E$, 11 Sept 1997, E. Kutorga (WI 7410); VILNIUS CITY, Verkiai forest, $54^{\circ}45'N$ $25^{\circ}17'E$, 25 Jun 2001, A. Kazlauskienė (WI 4714).

HABITAT IN LITHUANIA: Ash dieback symptoms occur in most habitats where *F. excelsior* grows. The ascomata of *H. fraxineus* were registered in leaf litter in different forests, although most commonly in mixed deciduous stands. Ascomata were also recorded from parks and nurseries under diseased trees, but these records were not confirmed microscopically.

NOTES: The causal agent of ash dieback, pathogenic *H. fraxineus*, is an alien invasive ascomycete in Europe that is replacing the closely related saprobic

indigenous species, *H. albidus* (Queloz et al. 2011, 2012; McKinney et al. 2012; Baral et al. 2014; Gross et al. 2014). *Hymenoscyphus fraxineus* is well known from the characteristic disease symptoms (Gross et al. 2014) and molecular data obtained from both isolated fungal cultures and symptomatic ash tissues. In Lithuania, crown dieback of *F. excelsior* was first observed in 1996 and by 2001 it had already become widespread (Juodvalkis & Vasiliauskas 2002). The disease has devastated ash stands all over the country and currently is in its chronic phase (Pliūra et al. 2011, Bakys 2013, Lygis et al. 2014). Initially, its primary causal agent was not properly diagnosed (Lygis et al. 2005, Vasiliauskas et al. 2006). The fungus was correctly identified only in 2008 when DNA sequence of a fungus (labelled as *Hymenoscyphus* sp. 970 by Lygis et al. in 2005) isolated in 2001 from a root collar of declining *F. excelsior* was assigned to *H. fraxineus* (GenBank AY787704, as *Chalara fraxinea*).

Since 1997, stromatized *F. excelsior* leaf petioles with numerous ascomata of *Hymenoscyphus* sp. collected in Lithuania have been deposited in herbaria BILAS and WI. The ascomata were first assigned to the saprobic *H. albidus* (Treigienė et al. 2010). Recently, Zhao et al. (2012) and Baral & Bemmam (2014) observed subtle microscopic differences in ascomatal structures between closely related *H. albidus* and *H. fraxineus* and underlined the importance of combining detailed morphological studies with molecular analysis in the *H. albidus* / *H. fraxineus* species complex. They reported that the presence or absence of croziers at the ascus base could distinguish *H. albidus* from *H. fraxineus*, differences that are strictly correlated with molecular characteristics. Therefore, we re-examined all herbarium specimens microscopically in order to clarify the status of the two *Hymenoscyphus* species in Lithuania. As the asci of all studied specimens had croziers, they were assigned to *H. fraxineus*. This suggests that there is no official proof of previous or current occurrence of *H. albidus* in Lithuania. It is worth mentioning that the Lithuanian collections of *H. fraxineus* specimens with ascomata (collected between 1997–2002) represent one of the earliest herbarium records of this fungus in Europe.

Leucocoprinus cepistipes (Sowerby) Pat., J. Bot. 3: 336. 1889.

Basidiomata growing in clusters. Pileus 2–6 cm diam, conic-campanulate when young, later convex to plano-convex, with an obtuse umbo. Surface dry, white to cream-colored, fibrillose, soon developing small white to cream-colored scales. Context white, soft, very thin. Lamellae free, narrow, white, becoming pallid to pale buff, edges white-floccose. Stipe 3–10 × 0.3–0.6 cm, cylindrical, hollow; base swollen, stipe surface above the annulus white and smooth, below – finely white-floccose, turning brownish when rubbed. Annulus fibrillose-membranous, white, with a somewhat crenate margin. Odor and taste mild,

not distinctive. Basidiospores ellipsoid to ovoid, smooth, hyaline, with a small germ pore, $6\text{--}11 \times 5\text{--}7 \mu\text{m}$. Basidia clavate, with 4 sterigmata, without basal clamp, $22\text{--}35 \times 10\text{--}12 \mu\text{m}$. Cheilocystidia clavate, sometimes rostrate, $25\text{--}60 \times 8\text{--}15 \mu\text{m}$. Pleurocystidia absent.

SPECIMENS EXAMINED: LITHUANIA, VILNIUS CITY, Valakupiai, $54^{\circ}44'N$ $25^{\circ}19'E$, 10 Jun 2005, V. Urbonas (BILAS 30747); Fabijoniškės, $54^{\circ}44'N$ $25^{\circ}15'E$, 28 May 2009, V. Kleizaitė (WI 7278).

HABITAT IN LITHUANIA: on soil in a greenhouse and in a flowerpot in a private apartment.

NOTES: *Leucocoprinus cepistipes* is of tropical origin, native to Asia and Africa; it has been introduced to Europe with a plant material and is apparently spreading (Vellinga 2001). In central and northern Europe it grows almost exclusively indoors (Pidlich-Aigner et al. 2002, Knudsen & Vesterholt 2008, Gierczyk et al. 2011); however in western and southern Europe the fungus has been found also outdoors on humus-rich soil, woodchips, compost or manure. In northern Poland, *L. cepistipes* was once recorded outdoors, growing on a horticultural waste dump (Gierczyk et al. 2011); however, there are no data of its overwintering. In Latvia (Riga city), the fungus was found in a lawn strip between a street and a sidewalk (Daniele & Meiere 2011; I. Daniele, personal communication). In Lithuania, *L. cepistipes* has been found exclusively indoors.

Ophiostoma novo-ulmi Brasier, Mycopathologia 115: 155. 1991.

No sexual and asexual fruiting structures of *O. novo-ulmi* were observed in Lithuania. Disease symptoms were in accordance with those provided by Anonymous (2009). Characteristics of the cultures coincided with those provided by Brasier (1991).

MATERIAL EXAMINED: (for geographical coordinates see Norkutė 2013).

subsp. *novo-ulmi*: LITHUANIA, BIRŽAI DISTR., Karajimiškis, from *Ulmus glabra*, May 2011 (LPM ON13); KAUNAS DISTR., Raudondvaris, from *U. glabra*, Nov 2011 (LPM ON43); KĖDAINIAI DISTR., Aristava, from *U. minor*, Jun 2011 (LPM ON14, ON15, ON18, ON19, ON21–ON34, ON36–ON39, ON42); PASVALYS DISTR., Gulbinėnai, from *U. glabra*, May 2011 (LPM ON10); Paberliai, from *U. glabra*, May 2011 (LPM ON11, ON12); PLUNGĖ DISTR., Kuliai, from *U. glabra*, Jun 2011 (LPM ON48–ON51); ROKIŠKIS DISTR., Dusetos, from *U. glabra*, Jun 2011 (LPM ON44–ON47).

subsp. *novo-ulmi* $\times$ subsp. *americana* hybrid: LITHUANIA, KĖDAINIAI DISTR., Aristava, from *Ulmus minor*, Jun 2011 (LPM ON16, ON17, ON20, ON35, ON40, ON41); PLUNGĖ DISTR., Kuliai, from *U. glabra*, Jun 2011 (LPM ON52, ON53); VILNIUS CITY, Verkių park, from *U. glabra*, 12 Jul 2010 (LPM ON3; GenBank KJ677112); VILNIUS DISTR., Verkšionys, from *U. glabra*, 19 Jul 2010 (LPM ON5, ON6); Dūkštos, from *U. glabra*, 19 Jul 2010 (LPM ON7, ON8).

HABITAT IN LITHUANIA: According to information provided by the Department of Forest Sanitary Protection at Lithuanian State Forest Service (personal communication), Dutch elm disease symptoms occur in most habitats where elms (*Ulmus* spp.) grow.

NOTES: *Ophiostoma ulmi* (Buisman) Nannf. s. str. caused the original Dutch elm disease epidemic in Europe and North America in the mid-1900's (Gibbs 1978). *Ophiostoma novo-ulmi*, a more aggressive pathogen of elms, largely replaced *O. ulmi* during the second half of the 20th century (Brasier 1991). Seemingly, *O. ulmi* s. str. is already absent in most of the European countries as no recent data on occurrence of this species is available. *Ophiostoma novo-ulmi* has two subspecies – *O. novo-ulmi* Brasier subsp. *novo-ulmi* (Eurasian, EAN) and *O. novo-ulmi* subsp. *americana* Brasier & S.A. Kirk (North American, NAN) (Brasier & Kirk 2001). The species is invasive in Europe: NAN was introduced from North America in 1960's and EAN spread across Europe from east to west at about the same time (Brasier & Gibbs 1973, Brasier 1990, 1996).

In Lithuania, the outbreak of Dutch elm disease and the causative agent *O. ulmi* s. str. were first recorded in mid-20th century (Žuklys 1958), but afterwards this pathogenic ascomycete was not observed. Our molecular identification of fungal isolates from wood of wilting elm branches collected in 2010–2011 from different regions of Lithuania has demonstrated that all isolates represent *O. novo-ulmi*. FIG. 1 shows the RFLP banding patterns of *CU* and *COL1* genes from seven Lithuanian *O. novo-ulmi* isolates digested with restriction enzymes *HphI* and *BfaI*; according to the *CU* and *COL1* gene fragment sizes (see Konrad et al. 2002), isolates LPM ON47–ON51 (lanes 1–5) correspond with subspecies EAN, while LPM ON52 and ON53 (lanes 6 and 7) correspond with EAN ×

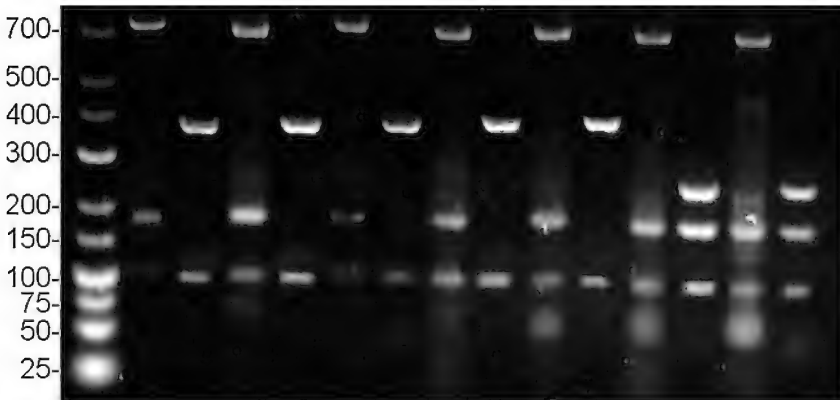


FIG. 1. RFLP banding patterns of cerato-ulmin gene *CU* from *Ophiostoma novo-ulmi* digested with restriction enzyme *HphI* (lane numbers labelled "c") and colony type gene *col1* digested with restriction enzyme *BfaI* (lane numbers labelled "l") after separation on a 2% agarose gel. Lane numbers represent seven *O. novo-ulmi* isolates: 1 = ON47; 2 = ON48; 3 = ON49; 4 = ON50; 5 = ON51; 6 = ON52; 7 = ON53 (see also Norkutė 2013). M = DNA marker, Thermo Scientific GeneRuler Low Range DNA Ladder (Thermo Fisher Scientific Inc.).

NAN hybrid. On the basis of similar RFLP patterns from all 49 Lithuanian *O. novo-ulmi* isolates, 36 were identified as EAN and 13 as EAN × NAN hybrid (Norkutė 2013). Our results suggest that *O. novo-ulmi* has replaced *O. ulmi* s. str. in Lithuania.

Stropharia rugosoannulata Farl. ex Murrill, Mycologia 14: 139. 1922.

Basidiomata numerous, growing solitary or gregarious. Pileus 5–15 cm diam, convex to broadly convex, surface dry, smooth, wine red to reddish brown, or pale brownish, sometimes cracked in old age (both color types were present in the Lithuanian sample); the margin with partial veil remnants. Context white, not changing color upon exposure. Lamellae broadly adnate, gray when young, later gray blue, black violet, close. Stipe 7–15 × 3 cm, central, cylindrical to slightly clavate, brittle; surface above the annulus white and finely striate, surface below with longitudinal fibrils, which are whitish at first and later pale yellowish on a whitish background; base with white mycelial threads. Annulus compact, pendent, white; surface finely striate on its upper side; margin splitting causing annulus to become stellate. Odor pleasant, taste mild. Basidiospores brownish-violet, ellipsoid to ovoid, smooth, thick walled, with a germ pore, 10–13 × 7.5–9 µm. Basidia clavate, with 4 sterigmata, without basal clamp, 26–33 × 8–9.5 µm. Cheilocystidia ventricose, clavate or lageniform, with apical protrusion, not changing color in KOH, 30–45 × 11–22 µm. Pleurocystidia fusiform-ventricose, some also with contents not changing color in KOH, 28–50 × 10–13 µm. Pileipellis periclinal, composed of brown-pigmented, 5–13 µm thick hyphae; some septa with clamps.

SPECIMEN EXAMINED: LITHUANIA, VILNIUS DISTR., Gineitiškės, 54°44'N 25°11'E, 9 Oct 2011, V. Būda (BILAS 50367).

HABITAT IN LITHUANIA: on wood chips in a garden among shrubs of *Ribes* spp.

NOTES: The fungus has apparently been introduced to Lithuania with imported gardening woodchips, a substrate known as a rich source of alien macromycete species (Shaw & Kibby 2001). *Stropharia rugosoannulata* is a widely cultivated mushroom (although not in Lithuania) that has also recently been observed in natural and semi-natural environments elsewhere in Europe (Breitenbach & Kränzlin 1995, Knudsen & Vesterholt 2008). At least in central Europe, the species is likely to be ephemeral as it usually disappears with deterioration of its substrate (Voglmayr & Krisai-Greilhuber 2002). This hypothesis is supported by Shaw et al. (2004), who state that substrates like woodchips made of ornamental trees can sustain diverse albeit short-lived fungal communities.

This is the first record of *S. rugosoannulata* in the eastern Baltic region.

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Some stromatic pyrenomycetous fungi from northern Thailand — 2. *Annulohypoxyton* and *Ustulina*

LARISSA N. VASILYEVA^{1*}, STEVEN L. STEPHENSON² & KEVIN D. HYDE^{3,4}

¹*Institute of Biology & Soil Science, Far East Branch of the Russian Academy of Sciences,
Vladivostok 690022, Russia*

²*Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701, USA*

³*Institute of Excellence in Fungal Research & ⁴School of Science, Mae Fah Lung University,
Chiang Rai 57100, Thailand*

* CORRESPONDENCE TO: vasilyeva@biosoil.ru

ABSTRACT—Fourteen species of *Annulohypoxyton* and one species of *Ustulina* collected in northern Thailand are discussed. Only three of the species (*Annulohypoxyton bahnpadengense*, *A. bogoriense* comb. nov., *A. urceolatum*) have been reported previously from southeastern Asia. The other twelve species (*A. chiangmaiense*, *A. derelictum*, *A. dipteroearpi*, *A. maesaeense*, *A. microbovei*, *A. morisuspectum*, *A. neglectum*, *A. paratruncatum*, *A. planodiscum*, *A. pseudonitens*, *A. sordidum*, *Ustulina pseudozonata*) are proposed as spp. nov., described, and illustrated.

KEY WORDS—Ascomycota, taxonomy, Xylariaceae

Introduction

In our first paper in this series (Vasilyeva et al. 2012), we emphasized that Thailand is the portion of the Indo-Malayan center of biodiversity characterized by a unique assemblage of vascular plants and, consequently, many associated species of fungi. As numerous xylariaceous fungi are host-specific (Rogers 1979, Whalley 1985, 1996, Stadler 2011) and those within the Indo-Malayan center of biodiversity would be expected to be likewise, it can be difficult to trace host-fungus associations in tropical forests, where dead branches are mixed together on the forest floor and the tree (or trees) from which they have fallen are not easy to identify. Nonetheless, we anticipate that future research will reveal definite substrate preferences for southeastern Asian species for which associations are now unknown.

In attempting to follow a biogeographical approach to taxonomy, we made a preliminary assessment of *Annulohypoxyton* species that might be found in Thailand. Several species and varieties are known only from southeastern Asia (Ju & Rogers 1996, Ju et al. 2004, Fournier et al. 2010), including *Annulohypoxyton bahnphadengense* (Thailand), *A. bovei* var. *microsporum* (J.H. Mill.) Y.M. Ju et al. (China, Japan, Philippines, Taiwan), *A. discophorum* (Penz. & Sacc.) Y.M. Ju et al. (Indonesia), *A. elevatidiscum* (Y.M. Ju et al.) Y.M. Ju et al. (Taiwan), *A. ilanense* (Y.M. Ju & J.D. Rogers) Y.M. Ju et al. (Taiwan), *A. maeteangense* J. Fourn. & M. Stadler (Thailand), *A. microcarpum* (Penz. & Sacc.) Y.M. Ju et al. (Indonesia), *A. moriforme* var. *microdiscum* (Y.M. Ju & J.D. Rogers) Y.M. Ju et al. (Taiwan), and *A. urceolatum* (Philippines, Sri Lanka, and Taiwan). These were the first taxa checked when identifying our specimens collected in Thailand. Although we recorded only two species (*A. bahnphadengense* and *A. urceolatum*) again in 2011, we expect others will be represented by additional collections in the future.

There are a few other species reported from both southeastern Asia and the tropics of the western hemisphere, but their types and many other specimens are mostly from the eastern hemisphere. For example, *Annulohypoxyton nitens* (Ces.) Y.M. Ju et al., described originally from Malaysia, has been found also in China, Philippines, Sri Lanka, and Taiwan. Its occurrence in Thailand has been reported by Suwannasai et al. (2002, 2005), Thienhirun et al. (2003), Thienhirun & Whalley (2004), Phosri et al. (2008), and Okane et al. (2008). Records of *A. nitens* from Mexico and Puerto Rico (Ju & Rogers 1996), Argentina (Hladki & Romero 2009), and Brazil (Pereira et al. 2010b) may represent a different taxonomic entity (cf. Vasilyeva et al. 2012, Vasilyeva & Stephenson 2014). In contrast to the southeastern Asian concentration of *A. nitens*, the distribution of *A. stygium* (Lév.) Y.M. Ju et al. is centered primarily in the Caribbean Basin. Specimens reported from Indonesia and Malaysia as *Hypoxyton stygium* (e.g., Whalley & Whalley 2007) might be assigned to other species (such as *Hypoxyton bogoriense* and *H. stigmoideum* Ces.) described previously from these countries, and the unification of the three entities (*H. bogoriense*, *H. stigmoideum*, *H. stygium*) into a single species (Ju & Rogers 1996) might need further consideration. Collections from Thailand identified as *H. stygium* (Whalley et al. 1995, Suwannasai et al. 2002, 2005, Thienhirun et al. 2003, Tang et al. 2007, Phosri et al. 2008) might also represent different species.

Materials & methods

The specimens considered herein were collected in two provinces in northern Thailand: Chiang Mai (Mae Taeng District near the villages of Pha Deng and Mae Sae; and the Doi Suthep temple in the vicinity of the city of Chiang Mai) and Chiang

Rai (Doi Pui Mountain in the Muang District). Microscopic analyses were carried out using standard techniques. Material was mounted in 10% KOH for examination of stromatal pigments and dehiscence of the perispore, and Melzer's iodine reagent was used for examination of the ascus apical ring. Color designations follow Rayner (1970). Photographs of stromata and associated structures were taken using a Nikon D40x digital camera. Measurements were obtained from about 15 ascospores and 5 glomerate stromata to represent the range of variation. The material from Thailand is deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU).

Taxonomy

Annulohypoxyton bahnphadengense J. Fourn. & M. Stadler,

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Figs 1, 15a

SPECIMEN EXAMINED: THAILAND, CHIANG MAI PROVINCE, Mae Taeng District, Pha Deng village, Mushroom Research Centre, on wood, 19 June 2011, L. Vasilyeva (MFLU11-1165).

NOTE: *Annulohypoxyton bahnphadengense*, characterized by effused-applanate, dull black to shiny black stromata, yielding grayish sepia (106) pigments in 10% KOH, ostiolar discs 0.25–0.3 mm diam., ascospores $6.5\text{--}8 \times 3\text{--}3.5 \mu\text{m}$, with a faint straight germ slit spore-length, perispore dehiscent in 10% KOH, and described only recently by Fournier et al. (2010), was collected again in same general locality.

Annulohypoxyton bogoriense (Höhn.) Lar.N. Vassiljeva, S.L. Stephenson &

K.D. Hyde, **comb. nov.**

Figs 2, 15b

MYCOBANK MB 801406

= *Hypoxyton bogoriense* Höhn., Sitz. K. Akad. Wiss. Math.-Nat. Kl., Abt. 1, 118: 341. 1909.

STROMATA effused, rather plane, with mostly inconspicuous (sometimes conspicuous) perithecial mounds at the margins, surface brown or dark-reddish-brown to the naked eye but pinkish between the black ostiolar discs in direct light and under low magnification, shiny black around the individual perithecia, with citrine (13) to olivaceous buff (89) KOH-extractable pigments. PERITHECIA spherical, 0.3–0.4 mm diam., ostioles papillate, encircled with a slightly concave or flat ostiolar discs 0.2–0.25 mm diam. ASCI not observed. ASCOSPORES light brown, ellipsoid-inequilateral, $5\text{--}7 \times 2.4\text{--}3 \mu\text{m}$, with a straight germ slit shorter than the spore length; perispore dehiscent in 10 % KOH.

SPECIMEN EXAMINED: THAILAND, CHIANG MAI PROVINCE, Mae Taeng District, Pha Deng village, Mushroom Research Centre, on wood, 24 June 2011, L. Vasilyeva (MFLU11-1166).

NOTES: *Hypoxyton bogoriense* was listed as a synonym of *Hypoxyton stygium* by Ju & Rogers (1996). Our illustration (FIG. 2) of the specimen from Thailand

shows the pinkish stromatal surface between the black ostiolar discs, which is similar to *A. atroroseum* (J.D. Rogers) Y.M. Ju et al. *Annulohypoxylon stygium* and *A. atroroseum* share many similar features, but mature stromata are usually shiny black in *A. stygium*, whereas those of *A. atroroseum* are rose-colored with a black ostiolar region (Ju & Rogers 1996). However, the mature stromata in our specimen from Thailand are not rose-colored; this tint is apparent only in direct light and under low magnification when photographed, and the actual color is dark brown or in accordance with Höhnelt's (1909) description of *Hypoxylon bogoriense* as being violet-brown and darkening with the age. Moreover, Rogers (1981) described *H. atroroseum* as having conspicuously sunken ostiolar discs, whereas those in our specimen are slightly concave and almost flat, thus slightly elevated above the surface. As Höhnelt indicated, the flat-convex tops of the perithecia, always at the margins but rarely in the middle parts of the stromata, stick out ("Scheitel der randständigen Perithecien stets, seltener auch der mittelständigen flachkonvex vorragend;" Höhnelt 1909, p. 341), and this condition corresponds to our specimen. Although *H. atroroseum* has been reported from Thailand (Suwannasai et al. 2005, Phosri et al. 2008), it was described originally from the west of central Africa (Gabon), and all specimens from Thailand might represent *H. bogoriense*, originally described from Indonesia.

Annulohypoxylon chiangmaiense Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,

sp. nov.

Figs 3, 15c

MYCOBANK MB 801393

Differs from *Annulohypoxylon bahnphadengense* by its larger ascospores and its citrine to olivaceous buff KOH-extractable pigments

TYPE: Thailand, Chiang Mai Province, Mae Taeng District, Pha Deng village, Mushroom Research Centre, on wood, 22 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1167).

ETYMOLOGY: refers to the province where the fungus was collected.

STROMATA effused-pulvinate, with perithecial mounds 1/2 to 1/4 exposed, surface at first brownish-olivaceous, then dark brown, with black granules immediately beneath surface and citrine (13) to olivaceous buff (89) KOH-extractable pigments. PERITHECIA spherical, 0.4–0.5 mm diam., ostioles papillate, encircled with flat ostiolar discs 0.3–0.35 mm diam. ASCI in the spore-bearing portions 70–80 × 4.5–5 µm, the stipes ≤60 µm, with a tiny apical ring bluing in Melzer's iodine reagent. ASCOSPORES brown, ellipsoid-inequilateral, 8.5–9.5 × 4–5 µm, with a straight germ slit slightly shorter than the length of the spore; perispore dehiscent in 10 % KOH.

NOTES: *Annulohypoxylon chiangmaiense* is superficially quite similar to *A. bahnphadengense*, also collected from the same site. However, the pigments



FIGS. 1–3. Stromata: 1. *Annulohypoxyton bahnphadengense*. 2. *A. bogoriense*. 3. *A. Chiangmaiense*.
Scale bars: 1, 2, 3a = 0.4 mm; 3b = 1.7 mm.

of *A. bahnphadengense* are grayish sepia and its light-brown ascospores (FIG. 15a) are 6.5–8.4 μm long (7.5 μm in average). It is also noteworthy that young portions of the stromata in *A. chiangmaiense* are brownish-olivaceous.

Annulohypoxylon derelictum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIG. 4

MYCOBANK MB 801394

Differs from other *Annulohypoxylon* species in lacking KOH-extractable pigments.

TYPE: Thailand, Chiang Mai Province, Muang District, T. Thasai, on dead branch of *Dipterocarpus* (?), in a dipterocarp forest, 30 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1168).

ETYMOLOGY: from the Latin *derelictus*, meaning ‘abandoned’ or ‘neglected’.

STROMATA effused-pulvinate, with perithecial mounds 1/4 exposed or inconspicuous, surface dull black, blackish granules immediately beneath surface, without apparent KOH-extractable pigments. PERITHECIA spherical, 0.3–0.5 mm diam., ostioles papillate and encircled with a flat or slightly concave disc 0.2–0.3 mm diam. ASCI not observed. ASCOSPORES brown to light brown, ellipsoid-inequilateral, 8–10.5 $\times$ 4.5–5.2 μm , with a straight germ slit almost the length of the spore; perispore dehiscent in 10% KOH.

NOTE: The most prominent feature of *A. derelictum* is the lack of KOH-extractable pigments, a condition not previously recorded for *Annulohypoxylon*.

Annulohypoxylon dipterocarpi Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 5, 15g

MYCOBANK MB801395

Differs from *Annulohypoxylon purpureonitens* by its smaller ostiolar discs and larger ascospores.

TYPE: Thailand, Chiang Mai Province, Muang District, T. Thasai, on dead branch of *Dipterocarpus* (?), in a dipterocarp forest, 30 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1169).

ETYMOLOGY: refers to the genus of the probable host plant, with the specimen collected in an almost pure dipterocarp forest.

STROMATA effused-pulvinate, with perithecial mounds 1/2 to 1/4 exposed or inconspicuous, surface black to the naked eye but brownish under direct light, blackish granules immediately beneath the surface, KOH-extractable pigments vinaceous purple (101). PERITHECIA spherical, 0.3–0.5 mm diam., ostioles papillate and encircled with a small concave disc about 0.1–0.2 mm diam. ASCI 70–80 $\times$ 5–6 μm long in the spore-bearing portions, the stipes 30–35 μm long, with an apical part not bluing in Melzer’s iodine reagent. ASCOSPORES brown to

light brown, ellipsoid-inequilateral, (8–)9–11(–12) $\times$ 4.5–5 μ m, with a straight germ slit the length of the spore; perispore dehiscent in 10% KOH.

NOTES: *Annulohypoxylon dipterocarpi* has vinaceous purple KOH-extractable pigments and is characterized by effused pulvinate stromata similar to *A. purpureonitens* (Y.M. Ju & J.D. Rogers) Y.M. Ju et al., known only from Brazil and Mexico (Ju & Rogers 1996); *A. purpureonitens* differs in having larger ostiolar discs (0.2–0.5 mm diam.) and smaller ascospores (6.5–10 μ m). The lack of a positive Melzer's iodine reaction in the asci is also diagnostic for *A. dipterocarpi*. When the perithecia are crashed in a mount, a mass of tiny ellipsoid spores 1.5–3 μ m long appears together with asci and ascospores, suggesting a conidial state within the stromata but probably instead a hyperparasitic anamorphic fungus.

Annulohypoxylon maesaeense Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 6a, 15h

MYCOBANK MB801396

Differs from *Annulohypoxylon moriforme* by its larger ascospores.

TYPE: Thailand, Chiang Mai Province, Mae Taeng district, near Mae Sae village, on the bark of an unidentified tree, 23 June 2011, S. Stephenson & T. Ko Ko (Holotype, MFLU11-1170).

ETYMOLOGY: from the Mae Sae village where the specimen was collected.

STROMATA glomerate, 3–5 mm diam., often confluent, with perithecial mounds 1/2 to 1/4 exposed, surface dark brown, blackish granules immediately beneath surface, with pale olivaceous but mostly slightly hazel (88) tinted KOH-extractable pigments. PERITHECIA spherical, $\leq$ 1 mm diam., ostioles papillate and encircled with a concave disc $\leq$ 0.3–0.35 mm diam. ASCI not observed. ASCOSPORES brown, ellipsoid-inequilateral, (8–)10–12(–12.5) $\times$ 4.5–5.5 μ m, with a straight or oblique germ slit extending the length of the spore or slightly shorter; perispore dehiscent in 10% KOH.

NOTES: Based on the key to taxa of *Hypoxylon* sect. *Annulata* (Ju & Rogers 1996), of the species characterized by having ascospores with a dehiscent perispore, ostiolar discs 0.2–0.5 mm diam, and dull brownish mature stromata), only two resemble *A. maesaeense*—*A. moriforme* (Henn.) Y.M. Ju et al. and *A. truncatum* (Schwein.) Y.M. Ju et al., both also reported from Thailand (Whalley et al. 1995, Suwannasai et al. 2002, Thienhirun et al. 2003, Phosri et al. 2008, Okane et al. 2008).

Annulohypoxylon moriforme, said to occur in the tropics and subtropics of both hemispheres (Ju & Rogers 1996), is distinguished from *A. truncatum* and *A. maesaeense* by its smaller ascospores (6–9 $\times$ 2.5–4 μ m). One variety, *A. moriforme* var. *microdiscum* from Taiwan, has larger ascospores comparable



FIGS. 4–6. Stromata: 4. *Annulohypoxylon derelictum*. 5. *A. dipterocarpi*. 6a. *A. maesaeense*.
6b. *A. truncatum* on *Quercus* (from Ouachita Mountains, Arkansas, VLA P-2156).
Scale bars: 4, 5 = 0.4 mm; 6a = 0.8 mm; 6b = 1 mm.

in size to those of *A. maesaeense*, but differs by having ostiolar discs smaller than 0.2 mm diam.

Annulohypoxyton truncatum (FIG. 6b) differs from *A. maesaeense* in having greenish olivaceous (90) to dull green (70) KOH-extractable pigments. Moreover, *A. truncatum* has been reported to inhabit *Quercus* in temperate regions, but species records actually have been indicated only from Mexico and the southeastern United States (North Carolina, Georgia, Louisiana, Massachusetts, Pennsylvania; Ju & Rogers 1996) as well as from Tennessee (Vasilyeva et al. 2007) and Arkansas and Texas (our unpublished data). *Annulohypoxyton truncatum* has also been recorded (as *Hypoxyton truncatum*) from Japan (Abe 1990, Abe & Doi 2000) and Korea (Velmurugan et al. 2007), not unexpected given that an appreciable number of pyrenomycetous fungi occur in both eastern North America and temperate regions of eastern Asia (Vasilyeva & Stephenson 2010). However, the eastern Asian material of *A. truncatum* needs to be reconsidered, in light of the redetermination of a specimen identified as “*A. truncatum*” from the Russian Far East (Vasilyeva 1998) to *A. orientale* Lar.N. Vassiljeva & S.L. Stephenson (Vasilyeva et al. 2013).

***Annulohypoxyton microbovei* Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,**

sp. nov.

FIGS 7, 15d

MYCOBANK MB801397

Differs from other species of the *Annulohypoxyton bovei* complex by its very small ostiolar discs.

TYPE: Thailand, Chiang Mai Province, vicinity of Chiang Mai city, near Doi Suthep temple, Huai Kok Ma, on dead branches of unidentified tree, 21 June 2011, L. Vasilyeva (Holotype, MFLU11-1171).

ETYMOLOGY: refers to the similarity of this fungus to the *Annulohypoxyton bovei* complex, albeit having smaller ostiolar discs.

STROMATA glomerate or effused-pulvinate, mostly confluent, with perithecial mounds 1/4 to 3/4 exposed, surface dark brown, blackish granules immediately beneath surface, with citrine (13) to hazel (88) tinted KOH-extractable pigments. PERITHECIA spherical, 0.5–0.7 mm diam., ostioles papillate and encircled with a slightly concave or flat disc 0.2–0.3 mm diam. ASCI not observed. ASCOSPORES brown, ellipsoid-inequilateral, 8–11 × 4.5–5.5 µm, with a straight germ slit spore length; perispore dehiscent in 10% KOH.

NOTES: The stromata and ostiolar discs in *A. microbovei* are similar to those in *A. bovei* (Speg.) Y.M. Ju et al., especially as illustrated by Miller (1961, Figs 158, 159). *Annulohypoxyton bovei* var. *microsporum*, widely distributed in southeastern Asia, is also known from China, Japan, Philippines, and Taiwan

(Ju & Rogers 1996) and shares a number of features (including ascospore size) with *A. microbovei* but differs in its larger (0.3–0.7 mm diam) ostiolar discs.

Annulohypoxylon morisuspectum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 8, 15e

MYCOBANK MB801398

Differs from *Annulohypoxylon moriforme* by its flat ostiolar discs and its brownish stromatal surface.

TYPE: Thailand, Chiang Mai Province, vicinity of Chiang Mai city, near Doi Suthep temple, Huai Kok Ma, on dead branches of unidentified tree, 21 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1172).

ETYMOLOGY: as compared with *A. moriforme*, with which it shares a number of similar features.

STROMATA glomerate, 1.5–2.5 mm diam., often confluent, with perithecial mounds 1/2 to 1/4 exposed, surface dark brown, blackish granules immediately beneath surface, with greenish to citrine (13) KOH-extractable pigments. PERITHECIA spherical, 0.4–0.5 mm diam., ostioles finely papillate and encircled with a flat disc 0.2–0.3 mm diam. ASCI 60–70 × 4–5 µm long in the spore-bearing portions, the stipes 35–50 µm long, with an apical ring bluing in Melzer's iodine reagent, discoid, 1.5–2 µm broad. ASCOSPORES brown to light brown, ellipsoid-inequilateral, 7.5–10 × 3–4.5 µm, with a straight germ slit slightly shorter than the length of the spore; perispore of at least some spores dehiscent in 10% KOH, but rarely.

NOTES: *Annulohypoxylon morisuspectum* resembles *A. moriforme* and *A. truncatum*, which are distinguished by their stromata with concave ostiolar discs of the *truncatum*-type; *A. moriforme* is further distinguished by the olivaceous shades of its stromatal surface (Ju & Rogers 1996).

Annulohypoxylon neglectum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 9, 15i

MYCOBANK MB801400

Differs from *Annulohypoxylon purpureopigmentum* by its smaller ascospores.

TYPE: Thailand, Chiang Mai Province, vicinity of Chiang Mai city, near Doi Suthep temple, Huai Kok Ma, on dead branches of an unidentified tree, 21 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1173).

ETYMOLOGY: refers to the small and inconspicuous, easily overlooked stromata.

STROMATA small, glomerate, 1.5–2 mm diam., often confluent, with perithecial mounds 1/3 to 1/4 exposed, surface dark brown, almost black to the naked eye, with a purplish tint under the dissecting microscope, blackish granules immediately beneath surface, with dilute bay (6) to purplish KOH-extractable



FIGS. 7–9. Stromata: 7. *Annulohypoxyton microbovei*. 8. *A. morisuspectum*. 9. *A. neglectum*.
Scale bars: 7 = 0.5 mm; 8 = 0.6 mm; 9 = 0.7 mm.

pigments. PERITHECIA spherical, 0.2–0.3 mm diam., ostioles rather coarsely papillate and encircled with a concave disc 0.15–0.2 mm diam. ASCI 40–50 × 3.5–4 µm long in the spore-bearing portions, the stipes 15–20 µm long, with an apical ring bluing in Melzer's iodine reagent, discoid, about 0.5–0.7 µm broad. ASCOSPORES light brown, ellipsoid-inequilateral, 4–6.3 × 2.5–3 µm, with a straight germ slit, perispore dehiscent in 10% KOH

NOTES: Despite its inconspicuous habit, *A. neglectum* possesses features uncommonly encountered in *Annulohypoxylon*, and their combination in the same taxon is noteworthy. Very small ascospores were reported for *A. atroroseum*, *A. ilanense* and *A. stygium*, but these species differ from *A. neglectum* by their widely effused stromata; in addition, *A. ilanense* differs by its brick colored KOH-extractable pigments (Ju & Rogers 1999), and *A. atroroseum* and *A. stygium* by their greenish olivaceous (90) to dull green (70) pigments (Ju & Rogers 1996).

Purplish KOH-extractable pigments are present in *A. purpureonitens* and *A. urceolatum*; the pigments in *A. neglectum* are similar but lack a vinaceous tinge (101 or 116). *Annulohypoxylon minutellum* (Syd. & P. Syd.) Y.M. Ju et al. [= *Hypoxylon cohaerens* var. *microsporum* J.D. Rogers & Cand.] has vinaceous to rusty KOH-extractable pigments (Hsieh et al. 2005), but this species does not have annulate ostioles. *Annulohypoxylon purpureopigmentum* Jad. Pereira et al. (Pereira et al. 2010a, Fig. 16) is characterized by purple vinaceous KOH-extractable pigments, but the ascospores (6.5–8(–10) µm long) are larger than those of *A. neglectum*. The appearance of glomerate stromata with large coarse papillae that almost fill up the concave discs is typical for *A. neglectum* and for *A. purpureopigmentum*, which seems the most similar western hemisphere species to *A. neglectum*.

Annulohypoxylon paratruncatum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 10a, 15f

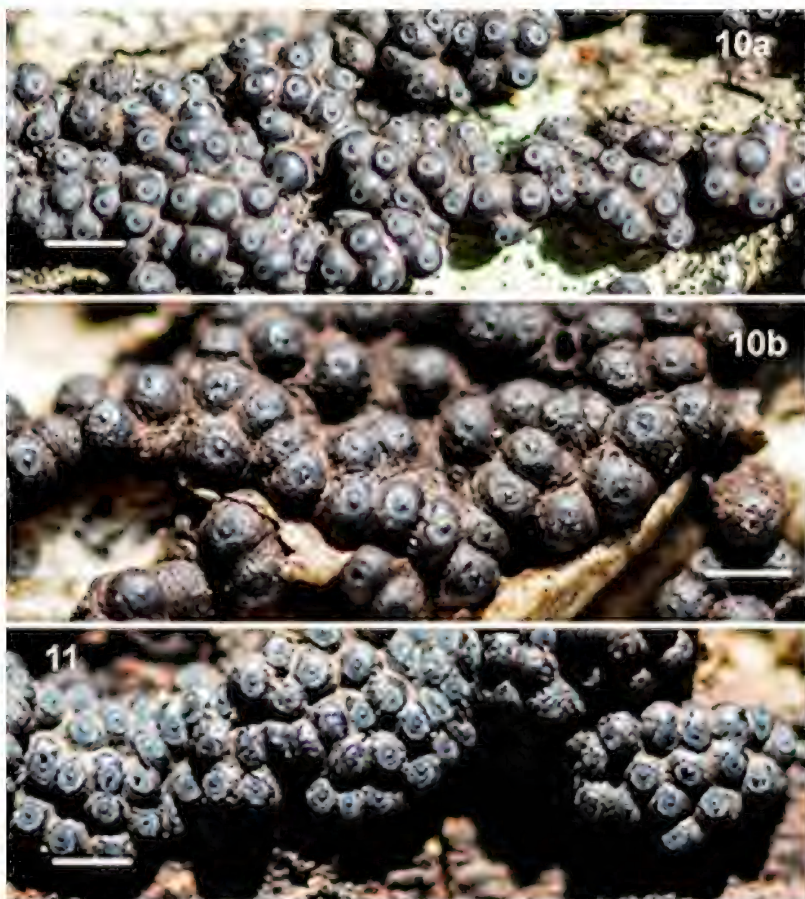
MYCOBANK MB801401

Differs from *Annulohypoxylon truncatum* by its smaller ostiolar discs and its citrine to olivaceous-buff KOH-extractable pigments.

TYPE: Thailand, Chiang Mai Province, Mae Taeng District, Pha Deng village, Mushroom Research Centre, on the bark, 19 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1174).

ETYMOLOGY: refers to the close similarity of the new fungus to *A. truncatum*.

STROMATA glomerate to effused-pulvinate, with perithecial mounds 1/2 to 1/4 exposed, surface dull black to the naked eye, dark brown under the dissecting microscope, blackish granules immediately beneath surface, with citrine (13) to olivaceous-buff (89) KOH-extractable pigments. PERITHECIA spherical, 0.3–0.4 mm diam., ostioles papillate and encircled with a concave disc about 0.2



FIGS. 10–11. Stromata: 10a. *Annulohypoxyton paratruncatum*.
10b. *A. truncatum* (from Ouachita Mountains, Arkansas, VLA P-2156). 11. *A. planodiscum*.
Scale bars: 10a = 1 mm; 10b = 0.9 mm; 11 = 1.3 mm.

(–0.25) mm diam. ASCI 60–80 × 4.5–5 µm long in the spore-bearing portions, the stipes 15–20 µm long, with an apical ring bluing in Melzer's iodine reagent, discoid, about 0.5 µm broad. ASCOSPORES brown to light brown, ellipsoid-inequilateral, 8–10.5 × 4–4.6 µm, with a straight germ slit shorter than length of the spore; perispore dehiscent in 10% KOH.

NOTES: Images of stromata show that *A. paratruncatum* (FIG. 10a) and *A. truncatum* (FIG. 10b) are superficially very similar; however, *A. truncatum*, which appears to have much larger perithecia and ostiolar discs, also differs by its greenish olivaceous (90) to dull green (70) KOH-extractable pigments.

Annulohypoxyton planodiscum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 11, 15j

MYCOBANK MB801402

Differs from *Annulohypoxyton elevatidiscum* by its glomerate stromata and flat ostiolar discs.

TYPE: Thailand, Chiang Mai Province, Muang District, Doi Pui Mountain, on the trunk of an unidentified tree, 30 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1175).

ETYMOLOGY: refers to the mostly flat ostiolar discs.

STROMATA glomerate (seemingly effused-pulvinate when confluent), with perithecial mounds 1/2 to 1/4 exposed, surface dull black (sometimes overlaid with a white substance, blackish granules immediately beneath the surface), with greenish-olivaceous (90) KOH-extractable pigments. PERITHECIA spherical, 0.3–0.4 mm diam., ostioles papillate and encircled with a flat disc 0.2–0.3 mm diam. ASCI 60–80 × 4.5–5 µm in the spore-bearing portions, the stipes 15–20 µm long, with an apical ring bluing in Melzer's iodine reagent, discoid, about 0.5 µm broad. ASCOSPORES brown to light brown, ellipsoid-inequilateral, 9–11.5 × 4–5 µm, with a straight germ slit spore extending the length of the convex side of the spore; perispore dehiscent in 10% KOH.

NOTES: The combination of several key characters in the type of *A. planodiscum*—greenish olivaceous KOH-extractable pigments, a 0.2–0.3 mm diam ostiolar disc, a straight germ slit on the convex side of the ascospores, and a perispore dehiscent in 10% KOH—are also found in *A. elevatidiscum* (Ju et al. 2004), which differs by its slightly larger ascospores and regularly effused-pulvinate stromata. Although some *A. planodiscum* discs appear to be slightly convex, the majority are flat, with its stromata initially glomerate and only later confluent, like those of *A. truncatum*.

Annulohypoxyton pseudonitens Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 12, 15l

MYCOBANK MB801403

Differs from *Annulohypoxyton nitens* by its smaller ascospores and its smaller ostiolar discs.

TYPE: Thailand, Chiang Mai Province, vicinity of Chiang Mai city, near Doi Suthep temple, Huai Kok Ma, on dead branches of an unidentified tree, 21 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1176).

ETYMOLOGY: refers to the similarity of this species with *Annulohypoxyton nitens* in a number of features.

STROMATA effused-pulvinate, with perithecial mounds 1/2 to 1/4 exposed, surface dull to shiny black in some parts, blackish granules immediately beneath the surface, with citrine (13) KOH-extractable pigments. PERITHECIA

spherical, 0.3–0.5 mm diam., ostioles papillate and encircled with a concave disc about 0.2 mm diam. ASCI 60–65 × 4–4.5 µm in the spore-bearing part, stalks 20–25 µm long, with an apical ring bluing in Melzer's iodine reagent, discoid, about 0.5 µm broad. ASCOSPORES brown to light brown, ellipsoid-inequilateral, 5–7.5 × 2.5–3 µm, with a straight or slightly oblique germ slit shorter than the length of the spore; perispore dehiscent in 10% KOH.

NOTES: *Annulohypoxyylon pseudonitens* resembles *A. nitens* in having shiny black mature stromata, somewhat exposed perithecial mounds, smaller ascospores, and greenish tinged KOH-extractable pigments. However, the ascospore length in *A. pseudonitens* does not exceed 7.5 µm, overlapping with the lower range cited for *A. nitens* (6.5–10(–11) µm long; Ju & Rogers 1996). As *A. nitens* has been reported previously from Thailand (Suwannasai et al. 2002, 2005, Thienhirun et al. 2003, Okane et al. 2008, Phosri et al. 2008), we first assigned our specimen to *A. nitens*. However, the type specimen of *A. nitens* [≡ *Rosellinia nitens* Ces.], treated as a synonym of *Hypoxyylon truncatum* (Schwein.) J. Mill. (Miller 1961), is cited as having 8–12 µm long ascospores (cf. Saccardo 1882, Miller 1961). In addition Hladki & Romero (2009) cite longer (8–9 µm) ascospores for a specimen of *A. nitens* from Argentina. The material of *A. nitens* from China and Philippines, identified previously as *Hypoxyylon bovei* var. *microsporum*, also has 8–10 µm long ascospores (Miller 1961, Ju & Rogers 1996), exceeding the spore length of *A. pseudonitens*.

Annulohypoxyylon bovei and its variety differ from *A. pseudonitens* by their mainly glomerate stromata.

Annulohypoxyylon sordidum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov. FIGS 13, 15k
MYCOBANK MB801404

Differs from *Annulohypoxyylon moriforme* by its effuse stromata and pale fawn KOH-extractable pigments.

TYPE: Thailand, Chiang Mai Province, Mae Taeng District, Pha Deng village, Mushroom Research Centre, on wood, 20 June 2011, L. Vasilyeva (Holotype, MFLU11-1177).

ETYMOLOGY: derived from the Latin *sordidus*, meaning 'dirty-looking'.

STROMATA effused-pulvinate, with perithecial mounds 1/2 to 1/4 exposed, surface dull, dark brown, blackish granules immediately beneath surface, with dilute fawn (87) KOH-extractable pigments. PERITHECIA spherical, 0.3–0.4 mm diam., ostioles papillate and encircled with a tiny concave disc up to 0.1 mm diam. ASCI 80–85 × 4.5–5 µm in the spore-bearing part, stalks 15–20 µm long, with an apical ring bluing in Melzer's reagent, discoid, about 0.5–0.7 µm broad. ASCOSPORES brown to light brown, ellipsoid-inequilateral, 9–11(–12) ×



FIGS. 12–14. Stromata: 12. *Annulohypoxyton pseudonitens*. 13. *A. sordidum*. 14. *A. urceolatum*.
Scale bars: 12, 13 = 0.6 mm; 14 = 1.4 mm.

4–5.5 μm , with a straight germ slit slightly shorter than the length of the spore; perispore infrequently dehiscent in 10% KOH.

NOTES: *Annulohypoxyton sordidum* has very small ostiolar discs similar to those of *A. moriforme* var. *microdiscum*, but both *A. moriforme* varieties are characterized by basically glomerate and blackish stromata with olivaceous shades, as well as greenish olivaceous (90) to dull green (70) KOH-extractable pigments.

The stroma of *A. sordidum* illustrated in FIG. 13 is somewhat similar to that of *A. pyriforme* (Y.M. Ju & J.D. Rogers) Y.M. Ju et al. (Ju & Rogers 1996, Fig. 14G) in having rather prominent perithecial mounds and small ostiolar discs. *Annulohypoxylon pyriforme* differs in its slightly smaller ($8\text{--}10 \times 3.5\text{--}4\text{ }\mu\text{m}$) pyriform ascospores, a perispore that is always indehiscent in 10% KOH, and dull green to dark green KOH-extractable pigments. *Annulohypoxylon sordidum* may represent a western hemisphere counterpart of *A. pyriforme*, known only from Venezuela (Cerro de la Neblina).

Annulohypoxylon urceolatum (Rehm) Y.M. Ju, J.D. Rogers & H.M. Hsieh,
Mycologia 97: 861. 2005.

FIG. 14

SPECIMEN EXAMINED: Thailand, Chiang Mai Province, Muang District, Doi Pui Mountain, on the branch of an unidentified tree, 30 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1178).

NOTES: *Annulohypoxylon urceolatum* is described in Ju & Rogers (1996, as *Hypoxylon urceolatum*). As indicated earlier, this species has been found only in southeastern Asia, but our record is the first for Thailand. We observed all the main features of this species such as the vinaceous purple KOH-extractable pigments, ascospores that are $9\text{--}14 \times 3.5\text{--}4.5\text{ }\mu\text{m}$, and a perispore that in our specimen is indehiscent in 10% KOH.

Key to the species of *Annulohypoxylon* considered in this paper

(New species indicated in bold font)

1. Stromata without apparent KOH-extractable pigments, effused-pulvinate;
ascospores $8\text{--}10.5\text{ }\mu\text{m}$ long *A. derelictum*
1. Stromata with KOH-extractable pigments 2
2. KOH-extractable pigments grayish-sepia, stromata widely effused,
ascospores $6.5\text{--}8.4\text{ }\mu\text{m}$ long *A. bahnphadengense*
2. KOH-extractable pigments of other colors 3
3. KOH-extractable pigments purplish tinged 4
3. KOH-extractable pigments of other colors 8
4. Stromata glomerate 5
4. Stromata widely effused 6
5. Ascospores $4\text{--}6.3\text{ }\mu\text{m}$ long *A. neglectum*
5. Ascospores $6.5\text{--}8\text{ }\mu\text{m}$ long *A. purpureopigmentum*
6. Ascospores $8\text{--}12\text{ }\mu\text{m}$ long 7
6. Ascospores up to $14\text{--}(17)\text{ }\mu\text{m}$ long *A. urceolatum*
7. Ostiolar discs $0.1\text{--}0.2\text{ mm}$ diam *A. dipterocarpi*
7. Ostiolar discs larger, $0.2\text{--}0.5\text{ mm}$ diam *A. purpureonitens*

8. KOH-extractable pigments greenish-olivaceous, dull green, citrine,
olivaceous buff, or hazel 9
8. KOH-extractable pigments brick colored *A. ilanense*
9. Stromata glomerate, often confluent and then becoming effused-pulvinate 10
9. Stromata flat and widely effused 18
10. Ostiolar discs of the *bovei* type 11
10. Ostiolar discs of the *truncatum* type 13
11. Ostiolar discs 0.2–0.3 mm diam 12
11. Ostiolar discs 0.3–0.7 mm diam *A. bovei* var. *microsporum*
12. Ascospores narrower (3–4.5 μ m), perispore almost indehiscent in 10% KOH
..... *A. morisuspectrum*
12. Ascospores wider (4.5–5.5 μ m), perispore dehiscent in 10% KOH ... *A. microbovei*
13. Ascospores 6–7(–9) μ m long *A. moriforme*
13. Ascospores 8–12 μ m long 14
14. Ostiolar discs do not exceed 0.2 mm diam 15
14. Ostiolar discs 0.2–0.4 mm diam 16
15. KOH-extractable pigments citrine to olivaceous-buff,
ascospores 8–10 μ m long *A. paratruncatum*
15. KOH-extractable pigments greenish olivaceous to dull green,
ascospores 10–12 μ m long *A. moriforme* var. *microdiscum*
16. KOH-extractable pigments close to hazel tint *A. maesaeense*
16. KOH-extractable pigments greenish-olivaceous to dull green 17
17. Ostiolar discs concave *A. truncatum*
17. Ostiolar discs flat *A. planodiscum*
18. Ostiolar discs of *bovei*-type *A. nitens*
18. Ostiolar discs of *truncatum* type 19
19. Ostiolar discs up to 0.1 mm diam *A. sordidum*
19. Ostiolar discs larger 20
20. Ostiolar disc 0.2 mm diam *A. pyriforme*
20. Ostiolar disc 0.3–0.4 mm diam 21
21. Ascospores 5–7 μ m long 22
21. Ascospores 8.5–9.5 μ m long *A. chiangmaiense*
22. Stromatal surface pinkish between black ostioles 23
22. Stromatal surface uniformly black 24
23. KOH-extractable pigments citrine to olivaceous-buff, ostiolar discs slightly concave
or almost flat *A. bogoriense*
23. KOH-extractable pigments greenish olivaceous to dull green, ostiolar discs
conspicuously sunken *A. atroroseum*

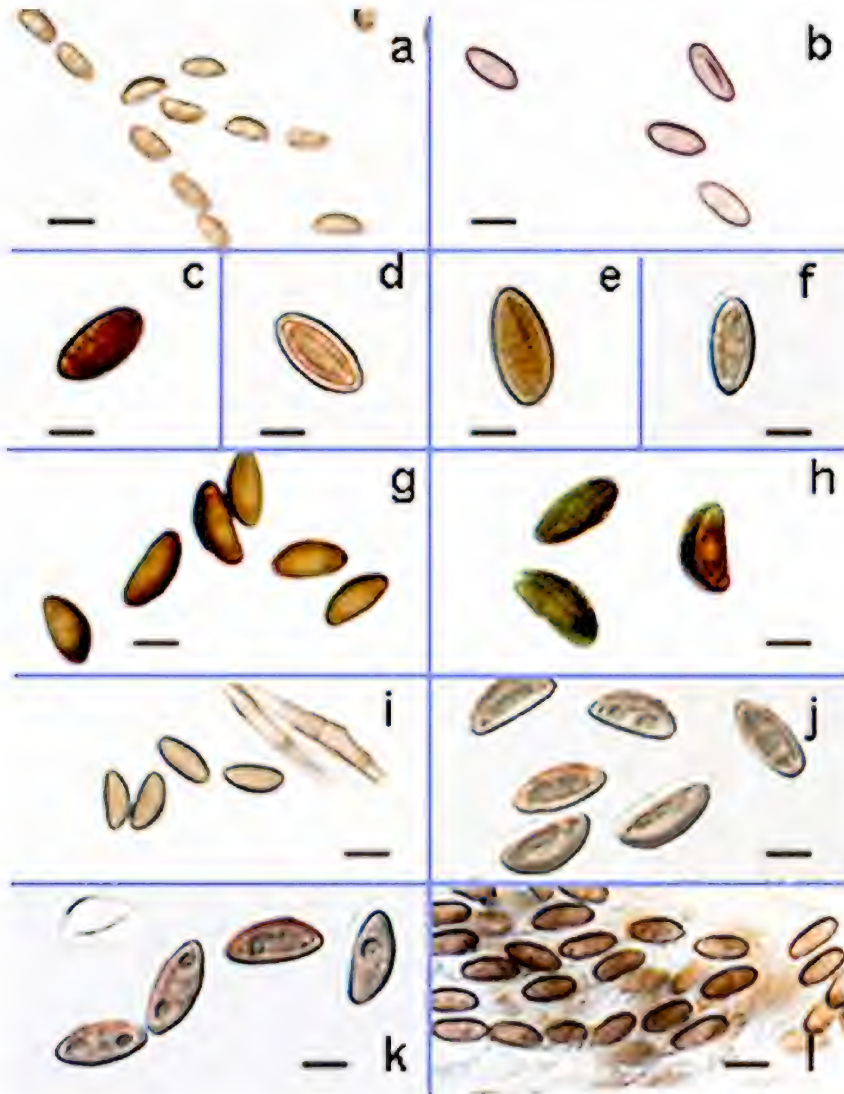


FIG. 15. Ascospores: a. *Annulohypoxyton bahnphadengense*. b. *A. bogoriense*. c. *A. chiangmaiense*. d. *A. microbovei*. e. *A. morisuspectum*. f. *A. paratruncatum*. g. *A. dipterocarpi*. h. *A. maesaeense*. i. *A. neglectum*. j. *A. planodiscum*. k. *A. sordidum*. l. *A. pseudonitens*. Scale bars: a = 7.5 μm ; b–d, f, i = 4 μm ; e = 3 μm ; g, h, j–l = 5 μm .

24. KOH-extractable pigments citrine *A. pseudonitens*
 24. KOH-extractable pigments greenish olivaceous to dull green *A. stygium*

Ustulina pseudozonata Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde,
sp. nov.

FIG. 16

MYCOBANK MB801405

Differs from *Ustulina zonata* by its larger ascospores and light-brownish stromata; and from *U. deusta* by its wider ascospores, longer germ slit, and light-brownish stromata.

TYPE: Thailand, Chiang Mai Province, Mae Taeng district, near Mae Sae village, on the bark of an unidentified tree, 23 June 2011, L. Vasilyeva (**Holotype**, MFLU11-1179).

ETYMOLOGY: refers to some similarity with *Ustulina zonata*.

STROMATA effused-pulvinate with the appearance of a flat elongated cake, 2.5–5 × 1.5–2.5 cm, light-brownish, with wavy and greyish margins, surface undulate, irregularly cracked, and very finely papillate, ostioles almost umbilicate. PERITHECIA tubular, 1–1.2 mm long, 0.4–0.6 mm wide. ASCI in the spore-bearing portions 180–200 × 12–14 µm, stipes up to 200 µm long, with an apical ring bluing in Melzer's iodine reagent, urn-shaped, 7–7.5 × 3–4 µm. ASCOSPORES brown, unicellular, fusoid-inequilateral, smooth, (27.5–)30–35 (–37.5) × 8–10 (–12) µm, with a straight germ slit extending almost the length of the spore.

NOTES: The name *Ustulina* is currently treated as a synonym of *Kretzschmaria* (Martin 1970, Læssøe 1994, Rogers & Ju 1998), but within *Kretzschmaria* sensu lato two taxonomic complexes can be clearly distinguished, the kretzschmarioid complex and the ustulinoid complex (Rogers & Ju 1998). Both complexes were accepted within a single genus based primarily on similar cultural features (Rogers & Ju 1998), but many other groups of xylariaceous genera (e.g., *Annulohypoxylon*, *Daldinia*, and *Hypoxylon* in one instance and *Entoleuca*, *Nemania*, and *Rosellinia* in another) known to share cultural features are nevertheless accepted as independent taxonomic entities (Stadler 2011).

We believe that *Ustulina* deserves recognition as a separate genus; among the ustulinoid taxonomic complex, the most closely related species to *U. pseudozonata* are *U. deusta* (Hoffm.) Lind and *U. zonata* (Lév.) Sacc. Rogers & Ju (1998) distinguish these two species in their key provided based on *U. deusta* having ascospores 27–35 (–38) × 7–9 µm, a germ slit much shorter than the spore length, and a northern temperate distribution vs. *U. zonata* having ascospores 21–34 × 8.5–12 µm, a germ slit that is either the length of the spore or nearly so, and a subtropical and tropical distribution. The specimen from northern Thailand has the range in ascospores length characteristic of *U. deusta* but a germ slit that fits *U. zonata*, and this combination is enough to characterize it as a separate species.

Ustulina zonata, described from Java, has been mentioned repeatedly as a pathogen that causes root decay of trees in southeastern Asia (Brooks 1915,

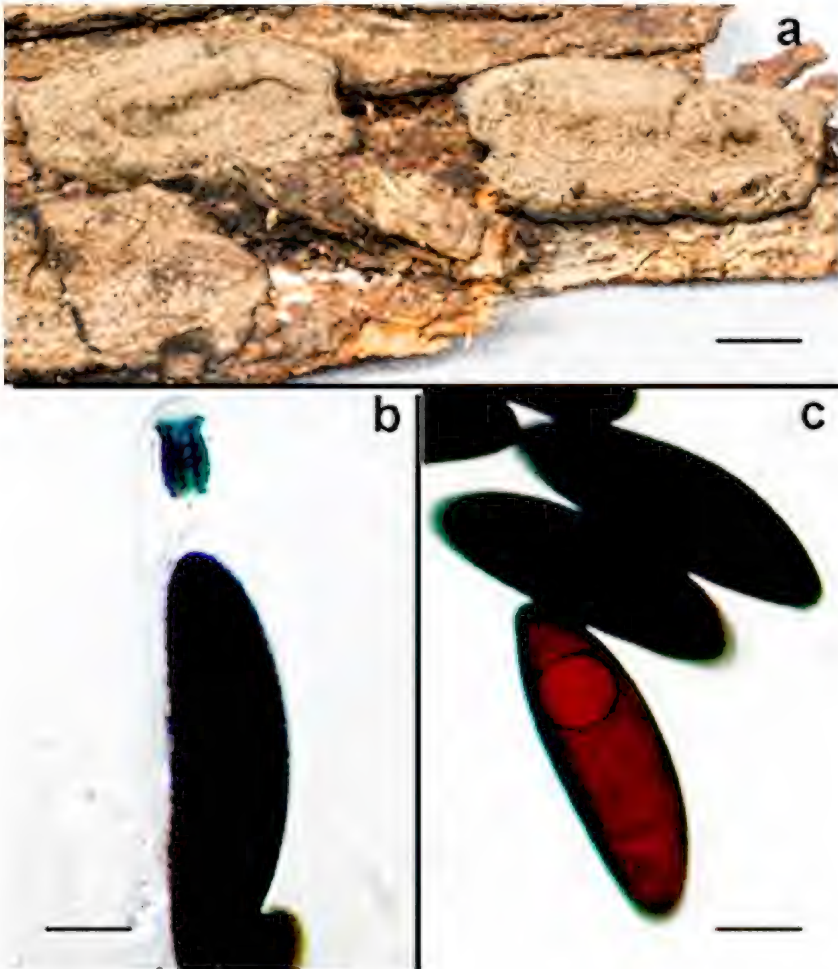


FIG. 16. *Ustulina pseudozonata*. a. Stromata (photo by Randy Darrah). b. Ascus top with an apical apparatus and an ascospore. c. Ascospores. Scale bars: a = 7 mm; b = 7 µm; c = 8 µm.

Petch 1928, Nandris et al. 1987, Muraleedharan & Baby 2007, Barthakur 2011). The only color illustration found on the Internet (<http://www.ava.gov.sg>) shows *U. zonata* as having carbonaceous fruit bodies hidden among grass and roots. The common name of the disease (charcoal stump root) seems to emphasize the black color of the stromata, although Petch (1928, p. 241) wrote that stromata usually referred to as representing *U. zonata* are “fairly frequently reddish on the surface, wholly or in part, but the majority of the specimens are, until

weathered, grey or purple-grey; when weathered, they are, of course, black.” He was unable to locate L  veill  s type of *U. zonata* but found L  veill  s specimen of “*U. deusta*”, which did not have zones on the fragment of a stroma, but had a reddish tinge. According to Petch, L  veill   distinguished *Ustulina zonata* from *U. vulgaris* (= *U. deusta*) by its reddish color and zoned surface. None of these features is mentioned by Rogers & Ju (1998), who indicate only that *U. zonata* has stromata, perithecia, ostioles, and asci similar to those found in *U. deusta*. In our specimen of *U. pseudozonata* from Thailand, the fully mature stromata are light brownish or sand-colored. This is the color reported for the maturing stromata in *Kretzschmaria milleri* J.D. Rogers & Y.M. Ju from the Caribbean Basin (French Guiana, Guyana, Mexico, Venezuela).

“*Ustulina zonata*” from southeastern Asia might represent a species complex waiting for separation into several separate taxa. Interestingly, almost all specimens listed under the description of *U. zonata* by Rogers & Ju (1998) are from the Caribbean Basin (Cuba, French Guiana, Guadeloupe, Mexico, Puerto Rico). Only one specimen from Taiwan was cited (Rogers & Ju 1998) for *U. zonata*, which differs from *U. pseudozonata* by its ascospores with pinched ends. Among the synonyms Rogers & Ju (1998) give for *Kretzschmaria zonata* is *Hypoxyton deustum* f. *madagascariensis* (Henn.) Hendr., which Dennis (1963) reported as having ascospores $20\text{--}28 \times 7\text{--}10\text{ }\mu\text{m}$ and which Rogers & Ju noted very likely represented *Kretzschmaria zonata*. The specimens from Thailand and Madagascar are not conspecific, primarily due to the spore size differences that ostensibly demonstrate the heterogeneity of the *Ustulina zonata* ‘complex.’ Given the economic importance of *U. zonata* as a pathogen of many trees and shrubs, further taxonomic investigations are warranted.

Discussion

In this paper, we have increased the number of examples of closely related species known from the Caribbean and Indo-Malayan centers of biodiversity (cf. Vasilyeva et al. 2012). As noted in earlier treatments of species from temperate latitudes of northeastern Asia and eastern North America (Vasilyeva & Stephenson 2010, 2011), the morphological differences between species in the western and eastern hemispheres are rather insignificant and sometimes pertinent to only a single character (e.g., a difference in ascospore size, of anamorphs, or in stromatal pigments). This enigma currently has no biogeographic explanation but does demand an accumulation of relevant examples to solve.

Specimens of six different *Annulohypoxyton* taxa (as *Hypoxyton bovei* var. *microsporum*, *H. moriforme*, *H. nitens*, *H. purpureonitens*, *H. stygium*, *H. stygium* var. *annulatum*) reported from Thailand by Suwannasai et al. (2002)

were said to differ from taxa described previously by Ju & Rogers (1996) due to a high level of morphological variation in the species involved. However, the differences might as easily be explained by the completely different species composition of the fungal assemblage found in Thailand. In fact, morphological variation within *Hypoxylon* and *Annulohypoxylon* is not very high, as both the same characters (stromatal pigments and shapes, dehiscent or indehiscent perispore, etc.) and the same ranges of variation (e.g., size of ascospores) repeat themselves infinitely (cf. Vasilyeva & Stephenson 2010) while combining differently with one another other for a particular taxon. Diversity lies not in morphological variation per se, but rather in the combinations of features characterizing different species.

Acknowledgments

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Taxonomic re-evaluation and phylogenetic position of *Hemibeltrania cinnamomi* within *Xylariales*

KUNHIRAMAN C. RAJESHKUMAR^{1*}, SAYALI D. MARATHE¹,
KARADKA MADHUSUDHANAN² & RAFAEL F. CASTANEDA-RUIZ³

¹National Fungal Culture Collection of India, Biodiversity and Palaeobiology Group,
Agharkar Research Institute, G.G. Agarkar Road, Pune, India

²Department of Botany, St. Albert's College, Cochin 18, Ernakulum, Kerala, India

³Instituto de Investigaciones Fundamentales en Agricultura Tropical 'Alejandro de Humboldt'
(INIFAT), Académico Titular de la Academia de Ciencias de Cuba,
Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P. 17200

*CORRESPONDENCE TO: rajeshfungi@gmail.com

ABSTRACT — *Hemibeltrania cinnamomi* associated with leaf blotch disease of *Cinnamomum malabattrum* (Lauraceae) is described and illustrated. Phylogenetic LSU and ITS sequence analyses support the fungus as a species of *Hemibeltrania* associated with other *Beltrania*-like genera. A synoptic table of *Hemibeltrania* species is provided.

KEY WORDS — asexual *Ascomycetes*, plant pathogen

Introduction

When revisiting *Beltrania* and related genera, Pirozynski (1963) introduced the genus *Hemibeltrania* (typified by *H. cinnamomi*) for a causal fungus of cinnamon leaf spot in Sierra Leone characterized by conidia described as ellipsoid to obovate and $12\text{--}16 \times 10\text{--}12\text{ }\mu\text{m}$. The phylogenetic placement of *Hemibeltrania* is unknown.

In December 2014, a survey was conducted to investigate microfungal diversity in the natural forests of Kaladi and the surrounding area within the southern Western Ghats, India. An unusual species of *Hemibeltrania* was discovered, which causes foliar blotches in *Cinnamomum malabattrum*. The present study aims to authenticate the species of *Hemibeltrania* using morphological characters and resolve the phylogenetic position of the genus using ITS and LSU sequence data.

Materials & methods

ISOLATES AND MORPHOLOGY—Conidia were directly isolated from the surface of a leaf spot and observed under a Nikon stereomicroscope (Model SMZ-1500 with Digi-CAM). Single conidial cultures were established on 2% Potato Dextrose Agar (PDA) and 2% Malt Extract Agar (MEA) plates (Crous et al. 2009). For morphotaxonomic studies and photomicrographs a Carl Zeiss Image Analyzer 2 microscope was used. Conidia and conidiophores were mounted in lactic acid cotton blue and measured using AxioVision REL.4.8 software, with 30 observations per structure. Colony characteristics in culture were studied on MEA and PDA (Crous et al. 2009). Herbarium specimens were deposited in Ajrekar Mycological Herbarium (AMH 9667), and the culture was accessioned and preserved in the National Fungal Culture Collection of India (NFCCI-3695), Agharkar Research Institute, Pune, India.

DNA EXTRACTION, AMPLIFICATION, AND PHYLOGENETIC ANALYSES—Colonies of *H. cinnamomi* were grown on MEA plates, and genomic DNA was extracted following the rapid salt-extraction method of Aljanabi & Martinez (1997). Fragments containing the region encoding the 28S nrDNA (LSU) and ITS 1-5.8S nrDNA-ITS 2 (ITS) were amplified using primer pairs LROR (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990) for LSU and primer pairs ITS4 and ITS5 (White et al. 1990) for ITS. PCR conditions, sequence alignments, and phylogenetic analyses followed the protocols of Crous et al. (2006). Thirty LSU sequences of the order *Xylariales* and an outgroup of two sequences (*Trichoderma* and *Lulworthia*) were downloaded from GenBank. Twenty-seven ITS sequences of the order *Xylariales* and an outgroup of two sequences (*Trichoderma* and *Lulworthia*) were downloaded also for phylogenetic analyses. The ITS and LSU regions were analysed by the Maximum Parsimony method with bootstrap (BS) analysis using 1000 replications in MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0). Sequence data were deposited in GenBank. Family placement of the sequences follows Shirouzu et al. (2010) and Hidayat et al. (2014).

Taxonomy

Hemibeltrania cinnamomi (Deighton) Piroz., Mycol. Pap. 90: 32 (1963) Figs 1, 2

SYMPTOMS: necrotic, reddish brown to brick brown, amphigenous, irregular.

ON THE NATURAL SUBSTRATE: CONIDIOPHORES distinct, single or slightly branched toward the apex, erect, straight or flexuous, cylindrical, slightly sinuate, 4–8-septate, smooth, dark brown at the base, pale brown toward the apex, thin-walled, $130\text{--}250 \times 3.5\text{--}6\ \mu\text{m}$, arising from a radially lobed basal cell, $10\text{--}11.5\ \mu\text{m}$ diam. CONIDIOGENOUS CELLS mono- and polyblastic, integrated or discrete, terminal, determinate or sympodial extended, denticulate; denticles cylindrical. Conidial secession schizolytic. CONIDIA solitary, limoniform, broadly ellipsoidal or obovate to irregular, rounded or obtuse at the apex, sometimes attenuate, subulate or rounded at the base with a small frill at the base, acrogenous becoming acropleurogenous after several sympodial extensions, unicellular, smooth, $12\text{--}25 \times 8\text{--}13\ \mu\text{m}$, hyaline, subhyaline to pale olivaceous.



FIG. 1. *Hemibeltrania cinnamomi* (AMH 9667).
a–f: Conidiophore branching. g–l: Denticulate conidiogenous cells. m–o: Conidiophore bases.
Bars: a–f = 50 μ m; g–o = 10 μ m.

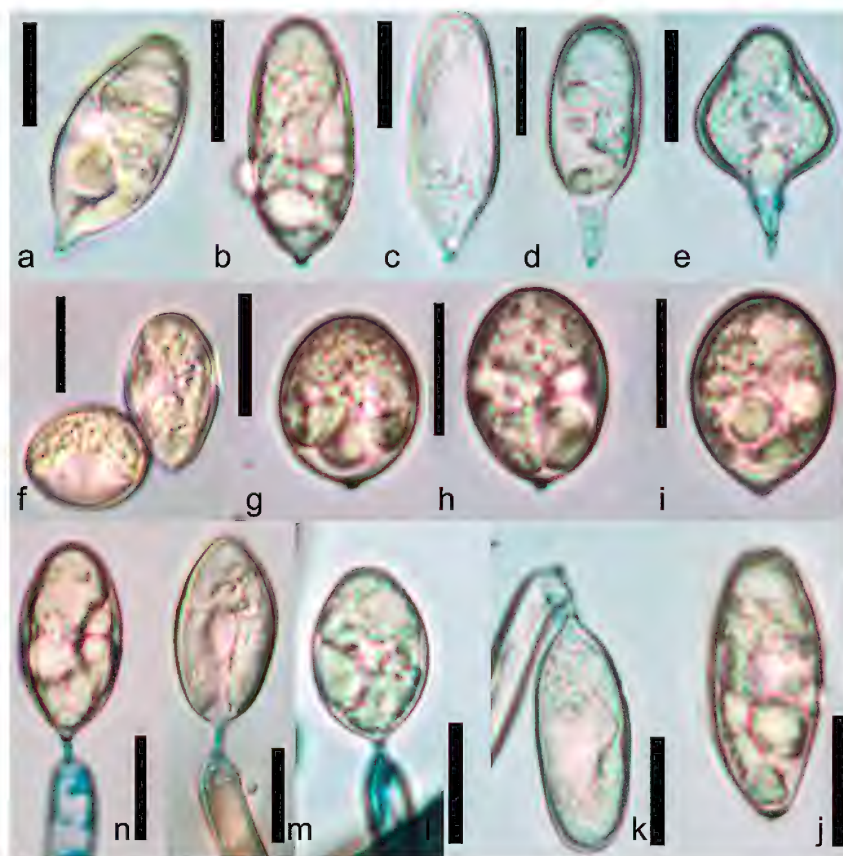


FIG. 2. *Hemibeltrania cinnamomi* (AMH 9667).
a–j: Conidia. k–n: Conidial attachments. Bars: 10 μ m.

Colonies on MEA slow to moderate growing, 35–45 mm diam after 7 days, grayish or mouse gray, later turning dark gray, velutinous, margin irregular, reverse blackish to blackish blue. Conidia 12–17 $\times$ 9.5–12 μ m.

SPECIMEN EXAMINED: INDIA. KALADI: Western Ghats, Kerala, 10°16'N 76°43'E, on leaves of *Cinnamomum malabattrum* (Burm. f.) J. Presl (*Lauraceae*) December 2014, K.C. Rajeshkumar & K. Madhusudhanan (AMH 9667; culture NFCCI 3695; Genbank KT119564, KT119565).

Phylogenetic analyses

LSU (KT119565): based on MegaBLAST search of GenBank LSU sequences, the closest hits are: *Xylaria* sp. (JQ862647.1, identities = 96%, gaps 0.2%);

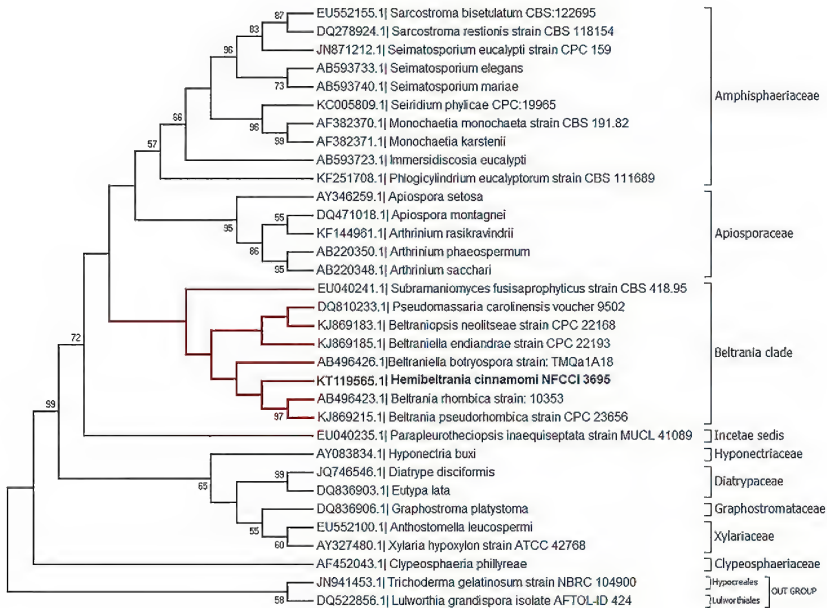


FIG. 3. LSU phylogenetic tree generated from Maximum Parsimony (MP) analyses of the aligned sequences of *Hemibeltrania cinnamomi* and allied taxa of the order Xylariales, with *Trichoderma* and *Lulworthia* as outgroup. Percentage bootstrap support ($\geq 50\%$) is shown on the branches. The MP tree was obtained using the Subtree-Pruning-Regrafting algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The bootstrap test was conducted with 1000 replicates.

Xylaria sp. (JQ862650.1, identities = 96%, gaps = 0.2%); and *Xylaria badia* (JQ862643.1, identities = 96%, gaps = 0.2%).

ITS (KT119564): A MegaBLAST of the ITS sequence showed that the closest hits are: *Hemibeltrania* sp. CL12WA (JQ621881.1, identities = 99%, gaps 0.4%); *Beltraniella endiandrae* CPC 22193 (KJ869128.1, identities = 93%, gaps = 3%); and *Beltrania pseudorhombica* CPC 23656 (KJ869158.1, identities = 92%, gaps = 4%).

The ITS and LSU phylogenetic analyses supported the genus *Hemibeltrania* as closely related to *Beltrania*, *Beltraniopsis*, *Beltraniella*, and *Subramaniomyces* and forming a unique monophyletic lineage in the Xylariales and closely allied to *Amphisphaeriaceae* and/or *Apiosporaceae* (Figs 3, 4).

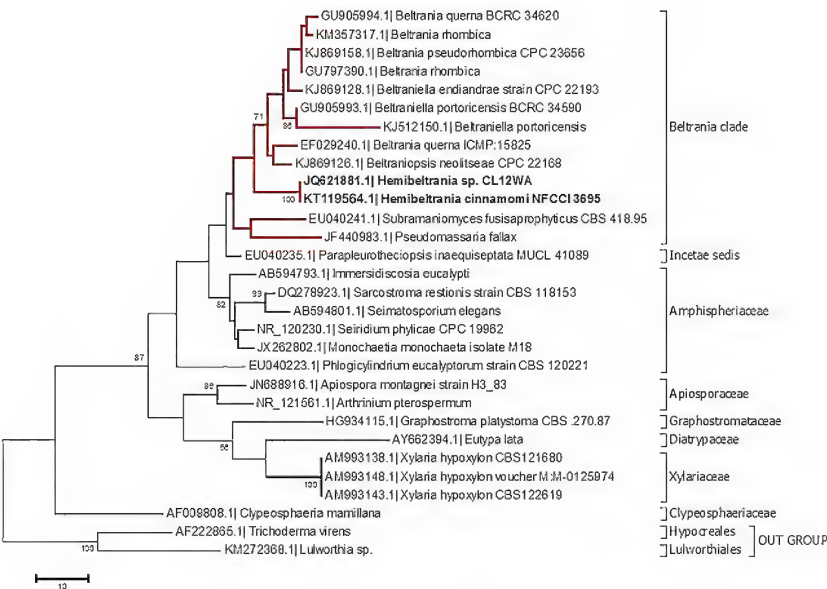


FIG. 4. ITS phylogenetic tree generated from Maximum Parsimony (MP) analyses of the aligned sequences of *Hemibeltrania cinnamomi* and allied taxa, with *Trichoderma* and *Lulworthia* as outgroup. Percentage bootstrap support ($\geq 50\%$) is shown on the branches. The MP tree was obtained using the Subtree-Pruning-Regrafting algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The bootstrap test has 1000 replicates.

Discussion

Hemibeltrania comprises 12 accepted species (MycoBank 2015; TABLE 1). Taxonomic study of the present collection revealed that in *H. cinnamomi*, conidial shapes and sizes are more variable than the original description: conidia are longer than $10\ \mu\text{m}$, while conidial shapes range from limoniform, obovate, and broadly ellipsoid to irregular. The symptomatology and other taxonomic characters, however, are identical to the type description given by Pirozynski (1963). Considering the variable conidial shape and size observed in AMH 9667, we propose a broad species concept for *H. cinnamomi* that might be expanded in the future based on re-examination and phylogenetic analysis of *H. nectandrae*, which shows some affinity with *H. cinnamomi*.

Earlier studies have shown that *Beltrania* and related genera (*Beltraniella*, *Beltraniopsis*, *Subramaniomyces*) are phylogenetically allied with *Amphisphaeriaceae*, *Xylariales* (Shirouzu et al. 2010, Hidayat et al. 2014). Shirouzu et al. (2010) also noted that *Beltraniella* anamorphs and

TABLE 1. Comparative morphology of accepted *Hemibeltrania* species

SPECIES	CONIDIA		CONIDIOPHORE SIZE (μm)
	SHAPE	SIZE (μm)	
<i>H. cinnamomi</i>	Obovoid/broadly ellipsoid/limoniform	12–25 $\times$ 8–13	130–250 $\times$ 3.5–6
<i>H. convallariae</i>	Ovoid	14–24 $\times$ 10–15	$\leq$ 2000 $\times$ 13–23
<i>H. cymbiformis</i>	Cymbiform	21–24 $\times$ 3–4.5	$\leq$ 140 $\times$ 2.2–4.5
<i>H. decorosa</i>	Fusiform	17–22 $\times$ 3.5	$\leq$ 250 $\times$ 4–6.5
<i>H. echinulata</i>	Ovoid	12–14 $\times$ 5.5–7	140–300 $\times$ 3.5–5
<i>H. laxa</i>	Obclavate/navicular	13–17 $\times$ 2–3	50–350 $\times$ 3–7
<i>H. malaysiana</i>	Ellipsoid/navicular	13–20 $\times$ 3–5	$\leq$ 400 $\times$ 4–7
<i>H. mitrata</i>	Mitriiform	14–17 $\times$ 4.5–5.5	35–85 $\times$ 3–4.5
<i>H. nectandrae</i>	Limoniform	15–23 $\times$ 10–13	$\leq$ 250 $\times$ 4–6
<i>H. ovalispora</i>	Obovoid	12–14 $\times$ 7	$\geq$ 750 $\times$ 4–6
<i>H. saikawae</i>	Clavate/cylindrical	14–19 $\times$ 2.5–3	10–155 $\times$ 2–3
<i>H. urbanodendri</i>	Ovoid/obovoid/ biconic/limoniform	12–25 $\times$ 6–10	55–106 $\times$ 4–6

Pseudomassaria teleomorphs are phylogenetically linked. Our phylogenetic analysis of *H. cinnamomi* does support *Hemibeltrania* within the *Xylariales*. However, the family in which it should be placed remains ambiguous.

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We are indebted to Dr. Elaine Malosso (Federal University of Pernambuco, Brazil) and Dr. De-Wei Li (The Connecticut Agricultural Experiment Station Valley Laboratory, USA) for reviewing this manuscript. Thanks are also due to Department of Science and Technology (DST), Government of India, New Delhi, for providing financial support and the Director, ARI, for providing facilities. RFCR is grateful to the Cuban Ministry of Agriculture and “Programa de Salud Animal y Vegetal” (project P131LH003033) for facilities. We acknowledge the assistance provided by Dr. P.M. Kirk and Drs. V. Robert and G. Stegehuis through the Index Fungorum and MycoBank websites. Dr. Lorelei L. Norvell’s editorial and Dr. Shaun Pennycook’s nomenclatural reviews are greatly appreciated.

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***Ramularia hydrangeicola* sp. nov. with distinctive traits on *Hydrangea serrata* f. *acuminata* in Korea**

JI-HYUN PARK & HYEON-DONG SHIN*

*Division of Environmental Science and Ecological Engineering, Korea University,
Seoul 02841, Korea*

*CORRESPONDENCE TO: hdshin@korea.ac.kr

ABSTRACT — A new species, *Ramularia hydrangeicola*, causing leaf spots was observed on *Hydrangea serrata* f. *acuminata*. Morphological characteristics and phylogenetic analyses support this fungus as a distinct new species. The new fungus is described, illustrated, and compared with *R. hydrangeae*, *R. hydrangeae-macrophyllae*, and *R. philadelphi*, three additional species on hosts of the *Hydrangeaceae*.

KEY WORDS — anamorphic *Mycosphaerella*, plant pathogen

Introduction

Ramularia is an anamorph-typified genus comprising hyphomycetous asexual morphs partly associated with sexual morphs of the genus *Mycosphaerella* (*Mycosphaerellaceae*). Based on the new rules of the ICN (Art. 59) and the new “one fungus one species” principles, *Mycosphaerella* has been reduced to synonymy with *Ramularia* since the type species of *Mycosphaerella*, *M. punctiformis*, forms a *Ramularia* anamorph, *R. endophylla*, and clusters with other *Ramularia* species (Verkley et al. 2004, Braun et al. 2013). The older name *Ramularia* has priority and is now applied as holomorph name for this monophyletic unit composed of asexual and sexual morphs (Braun et al. 2013, Wijayawardene et al. 2014). Most *Ramularia* species are phytopathogenic fungi that cause leaf spots, necrosis, or chlorosis; several species are economically important pathogens. Some *Ramularia* species are saprophytic or hyperparasitic (Braun 1998). *Ramularia* species are considered host-specific, like other fungi of the *Mycosphaerellaceae*. Species of *Ramularia*

and other allied genera have conventionally been morphologically delimited according to their host plant families and genera (Braun 1995).

Since 2002, a previously undescribed leaf spot has been observed on *Hydrangea serrata* f. *acuminata* during field surveys of cercosporoid fungi in Korea. The morphological characteristics of this fungus were quite different from those of other similar hyphomycetes observed on *Hydrangea* and were similar to those of *Ramularia* as described by Braun (1998). In this study, this novel species from Korea is described and illustrated on the basis of morphological characteristics and phylogenetic analyses. This species is compared with the three *Ramularia* species previously described on hydrangeaceous hosts: *R. hydrangeae*, *R. hydrangeae-macrophyllae*, and *R. philadelphica*.

Materials & methods

Fresh material and air-dried specimens were used for morphological examinations. The fungal structures were detached from the infected leaves and mounted in a few drops of distilled water or 3% KOH on a glass slide. These slides were examined by bright field- and differential interference contrast (DIC)-light microscopy, by using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements and a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) for imaging. Voucher specimens were deposited in the Korea University Herbarium, Seoul, Korea (KUS).

To obtain a pure isolate, infected tissue with abundant sporulation was excised and placed in a drop of distilled water on a glass slide. A loop of conidial suspension was streaked onto 2% water agar plates supplemented with 100 mg/L of streptomycin sulfate. After 2 days of incubation at 25°C, single conidial colonies were transferred onto potato dextrose agar (PDA). Two monoconidial isolates were deposited in the Korean Agricultural Culture Collection, National Institute of Agricultural Science, Jeonju, Korea (KACC).

The fungal mycelium was harvested from the culture on PDA and genomic DNA was extracted by means of a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). The internal transcribed spacer (ITS) region and the large subunit (LSU) of rDNA were amplified by using primers ITS1 and ITS4 (White et al. 1990) for ITS, and LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990) for LSU. The PCR amplicons were purified with the help of a QIAquick PCR purification Kit (Qiagen Inc.) and were sequenced by applying the MacroGen Sequencing Service (MacroGen, Seoul, Korea). The raw sequences were edited by means of DNASTAR version 5.05 (Lasergene, Madison, WI, USA). For phylogenetic analyses, all available ITS and LSU sequences that closely matched the extracted sequences were retrieved from the NCBI GenBank database. Sequence datasets for the two genomic loci were aligned on the base of an online version of MAFFT 7 (Katoh & Standley 2013). A phylogenetic tree was constructed by the neighbor-joining method using MEGA 6 (Tamura et al. 2013). Branches of the inferred tree were tested by a bootstrap analysis of 1000 replicates. Furthermore, a Bayesian inference analysis was performed according to MrBayes version

3.1.2 (Ronquist & Huelsenbeck 2003). Best-fit substitution models, which were obtained by applying MrModeltest version 2.2 (Nylander 2004), were used to ascertain the best nucleotide substitution model setting for each data partition. K80+I+G and GTR+I+G as nucleotide substitution models were used for ITS and LSU datasets. Branch support was calculated as posterior probability (PP) and neighbour-joining bootstrap (NJ-BS). The phylogenetic tree generated from the ITS sequence alignment was deposited in TreeBASE (<http://www.treebase.org/>) under accession number S17946.

Taxonomy

Ramularia hydrangeicola J.H. Park & H.D. Shin, sp. nov.

FIG. 1

MYCOBANK MB813449

Differs from *Ramularia hydrangeae*, *R. hydrangeae-macrophyllae*, and *R. philadelphica* in having conspicuous, hypophyllous, pinkish or reddish tinged caespituli and longer, wider conidia that are pinkish tinged, smooth, guttulate and mostly constricted at the septa.

TYPE: On living leaves of *Hydrangea serrata* f. *acuminata* (Siebold & Zucc.) E.H. Wilson (*Hydrangeaceae*): Korea, Yangpyeong, Okcheon-myeon, 37°34'45"N 127°27'28"E, 18 October 2007, H.D. Shin (Holotype: KUS-F23039; ex-type culture, KACC43597; GenBank KT265707, KT265708)

ETYMOLOGY: The epithet is derived from the host plant genus.

LEAF SPOTS amphigenous, scattered to confluent, angular to irregular, vein-limited, distinct, small, 1–5 mm diam., or up to 10 mm when confluent, at first visible as pinkish patches on the lower surface, pale greenish on the upper surface, indistinct discolorations, later becoming brown to dark brown on both sides. CAESPITULI hypophyllous, conspicuous, pinkish or with reddish tinge due to abundant fungal fructification. MYCELIUM internal; hyphae branched, hyaline or with faint pinkish tinge, 3–5 µm wide, occasionally with superficial secondary mycelium emerging through the substomatal cavity. STOMATA substomatal or sometimes intraepidermal. CONIDIOPHORES 3–8 in loose fascicles, emerging through stomata, solitary conidiophores arising from superficial hyphae, hyaline throughout or partly with pinkish tinge, 0–3-septate, simple, straight to curved, usually geniculate-sinuous, or 0–3 times geniculate near the apex, 12.5–40 × 2.5–5.0 µm. CONIDIOGENOUS CELLS integrated, terminal, intercalary, proliferating sympodially, 3.5–15 µm long; conidiogenous loci conspicuous, thickened and darkened, 1.2–2.1 µm diam. CONIDIA solitary or catenate, sometimes in short branched chains, cylindrical to obclavate-ellipsoid, hyaline or with pinkish tinge, smooth, guttulate, mostly uniseptate, occasionally 2–5-septate, constricted to non-constricted at the septa, obtuse to subobtuse at both ends, sometime gently attenuated towards each end, 18–45 × 3–7 µm; hilum minute, 1.2–1.8 µm diam., conspicuously thickened, darkened, and slightly protuberant. COLONIES on PDA attaining



FIG. 1. *Ramularia hydrangeicola*. A: symptoms on lower leaves of *Hydrangea serrata* f. *acuminata* with distinctive traits; B, C: close-up of the leaf lesions showing pinkish caespituli; D: conidiophores emerging through the substomatal cavity; E–G: conidiophores arising from superficial hyphae; H–J: catenate conidia; K–N: conidia ranging from hyaline to reddish tinged; O: 6-week-old colonies formed on potato dextrose agar (D–N = holotype, KUS-F23039; O = KACC43597). Scale bars: D–N = 20 μ m.

ca. 10 mm diam. after 6 weeks at 25°C under 12-h fluorescent light, velvety, pinkish to whitish, margins undulate, consisting of dense aerial mycelium and producing several droplets of slimy exudate. TELEOMORPH unknown.

ADDITIONAL SPECIMENS EXAMINED: on *Hydrangea serrata* f. *acuminata*: KOREA, JEJU, 14 Sep. 2002, H.D. Shin (KUS-F19005); 2 Nov. 2007, H.D. Shin (KUS-F23141); PYEONGCHANG, 27 Sep. 2002, H.D. Shin (KUS-F19080); 20 Sep. 2003, H.D. Shin (KUS-F19700); 9 Oct. 2008, H.D. Shin (KUS-F23778; culture, KACC44254; GenBank KT265709, KT265710); YANGPYEONG, 28 Aug. 2008, H.D. Shin (KUS-F23614); CHUNCHEON, 7 Oct. 2008, H.D. Shin (KUS-F23759); GAPYEONG, 13 Oct. 2011, H.D. Shin & J.H. Park (KUS-F26314); WONJU, 18 Sep. 2014, H.D. Shin & J.H. Park (KUS-F28177).

Results & discussion

Several mycosphaerellaceous fungi have been described on *Hydrangea* species, including *Cercospora*, *Pseudocercospora*, *Passalora*, *Phacellium*, and *Ramularia* spp. (Braun 1998, Crous & Braun 2003, Braun & Hill 2008, Farr & Rossman 2015). Our new species, *R. hydrangeicola*, is characterized by having mostly uniseptate conidia with thickened, darkened hila, either formed solitary or catenate, ranging from cylindrical to obclavate-ellipsoidal in shape and from hyaline to reddish tinged in color. These characteristics are consistent with the main characteristics of *Ramularia* (Braun 1998).

Three *Ramularia* species have been recorded on hosts of the family *Hydrangeaceae*: *R. hydrangeae* Y.L. Guo & U. Braun was described on *H. bretschneideri* from China (Braun 1998), based on material originally misdetermined as *R. saxifragae* (Guo 1993); *R. hydrangeae-macrophyllae* U. Braun & C.F. Hill was described on *H. macrophylla* from New Zealand (Braun & Hill 2008); and *H. philadelphi* Sacc., originally described from Italy, has been recorded on *Philadelphus* spp. from Europe, North America, and Asia (Braun 1998, Farr & Rossman 2015).

Ramularia hydrangeae differs from *R. hydrangeicola* in having inconspicuous, greyish white caespituli and hyaline, verruculose, shorter (8–35 µm), and narrower (2–5 µm) conidia (Braun 1998); *R. hydrangeae-macrophyllae* differs in having inconspicuous caespituli and shorter (4–18 µm), narrower (1.5–2.5 µm), and 0–1-septate conidia (Braun & Hill 2008); and *R. philadelphi* differs in having amphigenous, greyish white caespituli and hyaline, verruculose, shorter (8–20 µm), and narrower (2.5–5.0 µm) conidia (Braun 1998).

Phacellium hydrangeacearum U. Braun on *Jamesia americana* from the USA is another ramularioid fungus on a hydrangeaceous host, but easily distinguished by its synnematus conidiomata and verruculose conidia (Braun 1998).

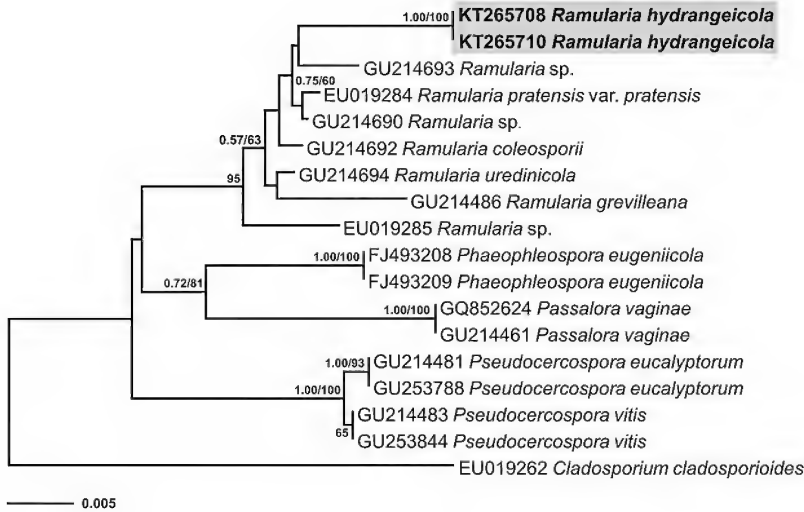


FIG. 2. LSU rDNA phylogenetic tree generated by a neighbor-joining method by using MEGA6 with 1000 bootstrap replications based on sequences of *Ramularia* and allied genera. Branch support is indicated above the branches (PP/NJ-BS). Sequences of *R. hydrangeicola* are shown in bold and their well-supported clade is highlighted in gray.

The holotype and paratype specimens of *R. hydrangeicola* yielded 1172-bp LSU and 495-bp ITS sequences. The LSU sequences were congruent between the two isolates, whereas their ITS sequences differed by three base-pairs. A GenBank BLAST search revealed that the LSU sequences had 99% similarity to GU214693 (*Ramularia* sp.), GU214690 (*Ramularia* sp.), and GU214692 (*R. coleosporii*); and the ITS sequences had 94% similarity to GU214693 (*Ramularia* sp.), EU019284 (*R. pratensis*), and KJ504787 (*R. decipiens*). Based on the molecular phylogenetic trees inferred from the neighbor-joining method and Bayesian analyses using ITS and LSU sequences, *R. hydrangeicola* formed an independent clade with high bootstrap values: PP = 1, NJ-BS = 100% (FIGS 2, 3). Moreover, the LSU phylogeny indicated that this species belongs in the genus *Ramularia*, and the ITS phylogeny revealed that it forms a separate lineage among *Ramularia* species. Although there are no comparable sequence data for *R. hydrangeae*, *R. hydrangeae-macrophyllae*, and *R. philadelphi*, *R. hydrangeicola* clearly differs morphologically from these species. Therefore, *R. hydrangeicola* is recognized as a new species based on the present morphological and molecular data.

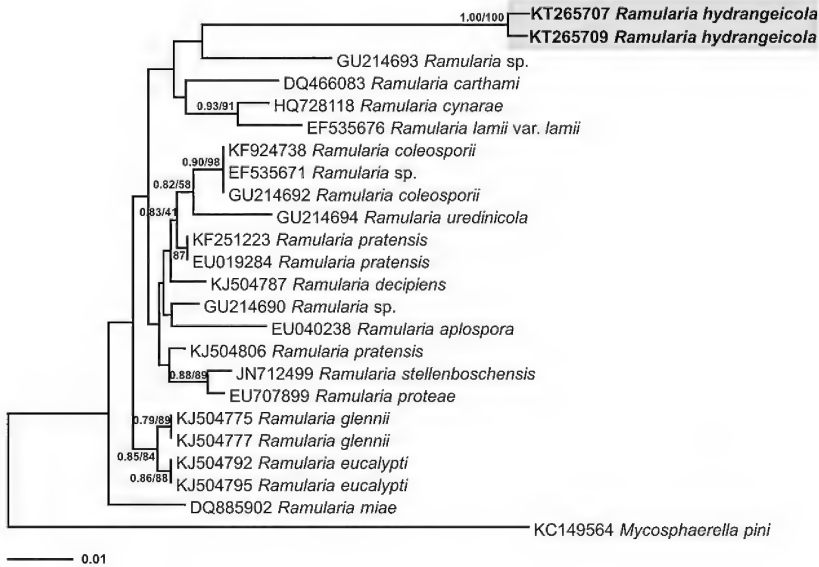


FIG. 3. ITS rDNA phylogenetic tree generated by a neighbor-joining method by using MEGA6 with 1000 bootstrap replications based on sequences among *Ramularia* species in GenBank database. Branch support is indicated above the branches (PP/NJ-BS). Sequences of *R. hydrangeicola* are shown in bold and their well-supported clade is highlighted in gray.

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Phylogenetic placement of the secotioid fungus *Araneosa columellata* within *Agaricus*

SCOTT T. BATES¹, ROBERT M. CHAPMAN², MELISSA B. ISLAM⁴,
ANNA SCHWABE⁴, ERIK C.P. WARDENAAR³ & VERA S. EVENSON⁴

¹Department of Biology, Purdue University Northwest, Westville, IN 46391, USA

²Las Cruces, NM 88012, USA

³The Bell Museum of Natural History, 10 Church St. SE, Minneapolis, MN 55455, USA

⁴Denver Botanic Gardens, 909 York St., Denver, CO 80206, USA

* CORRESPONDENCE TO: stbates@purdue.edu

ABSTRACT — Recently collected material was used to evaluate the phylogenetic position of the secotioid fungus *Araneosa columellata*. We amplified the nuclear ribosomal RNA gene (ITS1, ITS2, and LSU) and found *Ar. columellata* to be a member of the genus *Agaricus* (*Agaricaceae*, *Agaricales*), necessitating its transfer to that genus as *Agaricus columellatus* comb. nov. A detailed description is provided along with illustrations, and a lectotype is designated.

KEY WORDS — *Basidiomycota*, molecular phylogeny, Sonoran Desert, taxonomy

Introduction

DNA-based studies demonstrate multiple origins of gastroid (angiocarpic forms) and secotioid (stipitate angiocarpic forms often with reduced lamellae) species from within agaricoid (mushroom-forming) groups in the fungal phylogeny (Hibbett et al. 1997, Vellinga et al. 2003, Lebel & Syme 2012). Modern phylogenetic taxonomy (Queiroz & Gauthier 1992) stipulates that taxa be circumscribed as monophyletic clades, and this has necessitated the taxonomic reevaluation of these fungi. As a result, many familiar gastroid or secotioid taxa have been transferred into genera once reserved solely for agaricoid forms. For example, *Endoptychum agaricoides* was transferred to *Chlorophyllum* as *C. agaricoides* (Vellinga 2002), while *Endoptychum depressum* was transferred to *Agaricus* as *Ag. inapertus* (Vellinga et al. 2003).

Long (1941) described the monotypic secotioid genus *Araneosa* (type species: *Araneosa columellata*) within *Arachniaceae* (Coker & Couch 1928) from material collected at two localities in southern Arizona. Species within *Arachnion*, such as *A. album*, are superficially similar to *Ar. columellata*; however, *Araneosa* is distinguished primarily by its stipitate habit and the presence of a distinct columella within the gleba (Long 1941). Molecular phylogenetic studies have been needed to determine the taxonomic position of *Ar. columellata* (Moreno et al. 2007).

Several specimens of *Ar. columellata* collected by Long in the 1930–40s are deposited in institutional collections throughout the United States and Canada (BPI, FH, ILL, NY, NCU, UBC), many of which represent splits of the original type material (isotypes) in various stages of disintegration. An exceptionally well-preserved isotype (FIG. 1E) was located at the University of Illinois Herbarium, and this specimen is selected as the lectotype here. Specimens of this age are typically not desirable for DNA sequencing (Osmundson et al. 2013), and despite the need for new material, collections of this fungus have rarely been made (Esqueda 1998). On a recent excursion to the southwestern United States several fruiting bodies of *Ar. columellata* were collected. These specimens were morphologically identical to those described in the protologue (Long 1941) and yielded sufficient genomic DNA for Sanger sequencing of the nuclear ribosomal RNA gene (ITS1, ITS2, and LSU). This allowed us to examine the phylogenetic placement of *Ar. columellata* within the *Agaricales*.

Materials & methods

Original descriptions were made from material collected in the field, now deposited in the Fungal Collection of the Bell Museum of Natural History Herbarium at the University of Minnesota (MIN). An Olympus BH2 conventional bright field microscope was used in gathering the micro-morphological data on material first infiltrated with 95% aqueous ethanol and then mounted in water or 3% aqueous KOH. All measurements were made at 1000× using an ocular reticle with units calibrated by an optical micrometer. Spore statistics include: $\bar{x}$, the arithmetic mean of the spore length by spore width ($\pm$ SD) for n spores measured; Q , the quotient of spore length and spore width in any one spore, indicated as the range of variation in n spores measured; Q_m , the mean of Q values. Classical descriptive methodologies follow those published previously for angiocarpic fungi (Miller & Miller 1988), while color descriptions follow the system outlined in the Methuen Handbook of Colour (Kornerup & Wanscher 1967). Standard abbreviations for herbarium collections follow Index Herbariorum (Thiers 2016).

Genomic DNA was extracted following standard protocol of the Qiagen's DNeasy Kit, after first grinding glebal tissue and spores with a micro-pestle and then incubating this material in the kit buffer for 10 min. at 65°C. To amplify nuclear ribosomal RNA genes (ITS1, ITS2, and LSU), we used the primer pairs ITS1F/ITS4 (White et al. 1990, Gardes & Bruns 1993) and LR0R/LR5 (Vilgalys & Hester 1990, Bunyard et al. 1994).

PCR reactions were carried out under the following thermal cyclers conditions for LSU (with modifications for ITS in parentheses): 94°C for 3 min. for initial denaturation; followed by 35 cycles at 94°C for 30 sec. (1 min.), 54°C for 1 min., and 72°C for 1.5 min. (1 min.); then a final extension at 72°C for 4 min. (3 min.). PCR amplicons were then imaged in gel electrophoresis to visualize the presence and relative concentration of fungal DNA. Automated sequencing was carried out at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility.

Complementary DNA sequences were compiled and manually edited using MEGA 6.0 software suite (Tamura et al. 2013). Additional sequences of closely related taxa from within the *Agaricales* were culled from GenBank (<https://www.ncbi.nlm.nih.gov/>), with accession numbers cited in the phylogenetic tree (Fig. 2). MEGA 6.0 was used to align sequences and carry out the phylogenetic analysis. A total of 15 LSU nrRNA gene sequences was aligned using the MUSCLE algorithm (gaps were treated as complete deletions), which was used to carry out the maximum likelihood (ML) phylogenetic analysis (NNI heuristic method, with the K2+G nucleotide substitution model chosen under model selection analysis) and bootstrap testing (1000 replicates). The phylogenetic tree was rooted using *Psathyrella candolleana* (*Psathyrellaceae*, *Agaricales*) as outgroup for *Agaricus* (*Agaricaceae*, *Agaricales*; Matheny 2006).

Taxonomy

Agaricus columellatus (Long) R.M. Chapm., V.S. Evenson & S.T. Bates,
comb. nov.

MYCOBANK MB 809082

≡ *Araneosa columellata* Long, Mycologia 33(4): 353 (1941).

TYPE: USA, Arizona, Santa Cruz Co., 7 miles from Nogales on State Highway 89; open areas in mesquite-catclaw (*Prosopis-Acacia*) flats, elev. 3857 ft., 21.IX.1934, William H. Long (7937) and Victor O. Sandberg (the original type collection no. 7937 from Long's Herbarium now exists only as widely distributed isotypes: BPI 736388, BPI 736396, FH 488386, NY 449250!, NY 780610!, NY 780611!, NCU-F-0000798, UBC 371. **Lectotype designated here:** ILL 33529! (Fig. 1E)).

BASIDIOME 20–35 mm in diameter × 20–30 mm in height, secotoid, subglobose, depressed-globose to broadly ovoid or pyriform, furnished with a rudimentary stipe at the base that extends into the gleba as one unit to form a distinct columella, the entire unit often detaching completely and leaving a circular opening at the base of the peridial body; **STIPE-COLUMELLA UNIT** concolorous with the exoperidium, up to 30 mm long, with roughly half (~15 mm) of the unit protruding from the base of the basidiome and the upper half (~15 mm) inserted into the gleba, slightly bulbous at the base, up to 5 mm in diameter and even along the stipe portion, somewhat swollen at the point of insertion, and tapering toward the apex within the gleba as the columella, developing longitudinal striations and becoming more firm when dry, surrounded at the point of insertion by a thin layer of peridial tissue, this with striations radiating out from the central point of the stipe and resembling velar

FIGURE 1

tissue; PERIDIA slowly breaking apart to expose the gleba, often starting from the basal opening formed as the stipe-columella unit detaches. EXOPERIDIUM whitish to pale yellow (3A1–3), dull, coarsely to irregularly rugose overall, though glabrous at the surface, thin, firm and persistent, cracking apart or flaking off with age to expose portions of the endoperidium. ENDOPERIDIUM yellowish gray (3B2–4B2), dull glabrous, thin, firm and persistent, slowly breaking apart in conjunction with the exoperidium. GLEBA brown to grayish brown (6E3–5), composed of labyrinthiform, sinuous tramal tissue, forming numerous locules, the interior of which is a hymenial surface densely covered with brownish spore mass, and an exterior composed of grayish, loosely woven hyphal tissue, breaking apart into irregular shaped glebal fragments with age.

BASIDIOSPORES $4.0\text{--}5.6 \times 4.8\text{--}7.2 \mu\text{m}$ [$x = 4.8 \pm 0.5 \times 6.1 \pm 0.7 \mu\text{m}$, $Q_m = 1.3$, $n = 20$], subglobose to ovoid or occasionally irregular ovoid, smooth, brownish in water mounts; pedicel rudimentary or up to $4 \mu\text{m}$ in length; STERIGMATAL REMNANTS mostly absent from mounts. CAPILLITIUM absent. PERIDIA composed of thin-walled hyphal elements.

HABITAT: Terrestrial and found in soil amongst *Acacia*, palo verde (*Parkinsonia*), creosote (*Larrea*), mesquite (*Prosopis*) and jojoba (*Simmondsia*). *Agaricus columellatus* occurs at lower elevations within the Arizona Upland Subdivision of the Sonoran Desertscrub and Semidesert Grassland biotic communities (Brown 1994).

DISTRIBUTION: In the United States, known only from southern Arizona. Also reported from the state of Sonora in Mexico.

GENETIC DATA: Nuclear ribosomal RNA gene sequences for *Agaricus columellatus* (MIN 938394) have been deposited in GenBank (ITS: KJ912899; LSU: KJ912900).

ADDITIONAL SPECIMENS EXAMINED: USA, ARIZONA, GILA Co., AZ 288, north of Globe, $33.67^\circ\text{N } 110.96^\circ\text{W}$, alt. 707 m, in sand, amongst *Acacia*, palo verde, creosote bush, and jojoba, 30.VIII.2012, R.M. Chapman 1385 (MIN 938394); AZ 288, north of Globe, $33.67^\circ\text{N } 110.97^\circ\text{W}$, alt. 721 m, in sand, amongst *acacia*, palo verde, creosote bush, and jojoba, 1.IX.2012, R.M. Chapman 1388 (hb. Chapman). SANTA CRUZ Co., 7 miles north of Nogales, Arizona on Nogales–Tucson road, 30.IX.1939, William H. Long 8388 (paratype NY 2169336).

Results & discussion

Long (1941) described *Araneosa columellata* within *Arachniaceae*, stating “peridioles subglobose to irregularly oblong to angular, walls rather thick and firm, 90–268 microns by 134–402 microns, held loosely together by delicate interwoven hyphae, easily separable from the endoperidium, leaving its inner surface reticulate, the lamelliform arrangement of the peridioles often permitting the gleba to split into flakes which easily crumble into the

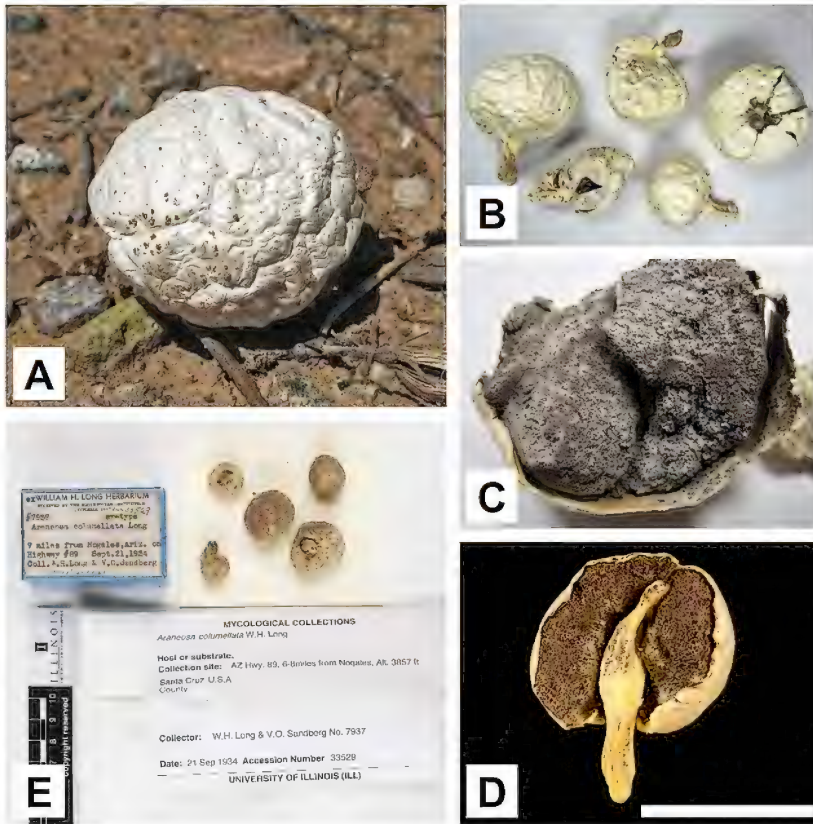


FIGURE 1. *Agaricus columellatus*. A. Field image of the gasterocarp (MIN 938394). B. Dried specimens (MIN 938394, bar = 20 mm). C. Exposed gleba of dried specimen (MIN 938394). D. Cross section through basidiome of dried specimen showing insertion of stipe-columella unit (MIN 938394; bar = 20 mm). E. Lectotype (ILL 33529).

individual peridioles.” Although our material was morphologically identical to Long’s (1941) concept of *Ar. columellata*, distinct peridioles did not form in the gleba. Inspection of authentic material from Long (NY 449250, NY 780610, NY 780611, NY 2169336, and the lectotype ILL 33529), revealed in these specimens the formation of irregularly shaped glebal fragments rather than discrete peridioles, which is consistent with our material (FIG. 1C). While the age of these specimens prevented us from confirming an exact match to the Long specimens by molecular means, on the basis of morphology, our specimens clearly represent a recent collection of *Ar. columellata*.

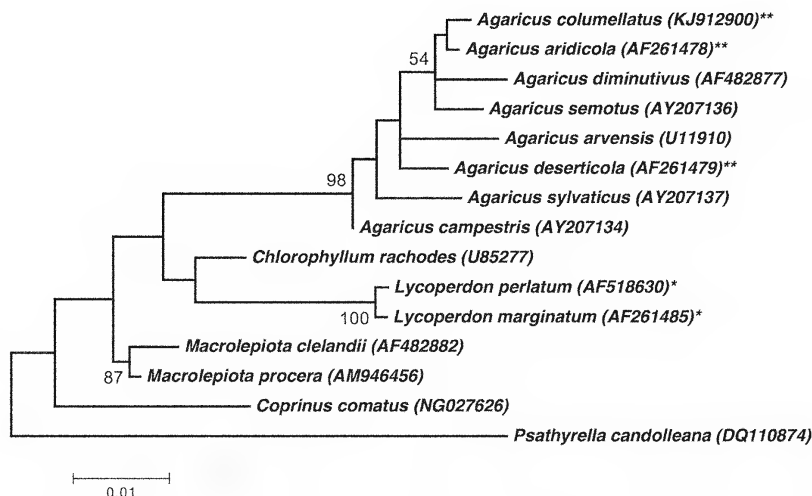


FIGURE 2. Molecular phylogeny (ML consensus tree) for the large subunit nuclear ribosomal RNA gene dataset showing the inclusion of *Agaricus columellatus* (MIN 938394), within *Agaricus* (*Agaricaceae*). Basidiome forms are agaricoid, unless otherwise indicated as gastroid (*) or secotiid (**). Branch lengths are shown at scale, with the bar representing the number of base pair changes along the branches, and nodes with $\geq 50\%$ bootstrap support are shown. The tree is rooted with *Psathyrella candolleana* (*Psathyrellaceae*), and the GenBank accession numbers are given for all taxa included in the tree.

Phylogenetic analysis of the LSU nrRNA gene alignment places our collection of *Ar. columellata* within *Agaricus* (*Agaricaceae*; FIG. 2); therefore, we transfer *Araneosa columellata* to this genus here as *Agaricus columellatus*. While our LSU tree (FIG. 2) shows *Ag. columellatus* as sister to *Ag. aridicola* ($\equiv$ *Gyrophragmium dunalii*), ITS sequence data (tree not shown) do not support a sister relationship for these two taxa, which share only 95% sequence similarity in the ITS region. Of the several Australian secotiid *Agaricus* species reported in the study of Lebel & Syme (2012), *Ag. columellatus* was most closely related to *Ag. wariatodes*; however, ITS sequence similarity between these two taxa was likewise very low (95%). Thus our study suggests yet another example of the independent origin of a unique secotiid fruiting body within the genus *Agaricus* and of the angiocarpic form more broadly across the *Agaricaceae*.

The distribution of *Ag. columellatus* is restricted to the desert regions of the southwestern U.S.A. and northern Mexico, with reports from the environs of Tucson and Nogales, Arizona, as well as Hermosillo in Sonora, Mexico (Long 1941, Esqueda et al. 1998, Moreno et al. 2007). The recorded range extends

a linear distance of approximately 400 km (north to south), while this study expands the distribution roughly another 170 km northward to the area of Globe, Arizona. This distribution essentially spans the western edge of the Sonoran Desert, though the area surrounding Nogales is technically considered outside of its boundary (Brown 1994). More extensive collecting in the area will almost certainly extend the range westward within the Sonoran Desert, and potentially eastward into the Chihuahuan Desert. The occurrence of *Ag. columellatus* within the arid-lands of North America reinforces the concept that selective pressure of xeric climates may have driven the evolution of angiocarpic forms from their agaricoid ancestors (Thiers 1984, Lebel & Syme 2012).

Acknowledgments

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Morphological traits, DELTA system, and molecular analysis for *Gibellula clavispora* sp. nov. from China

WAN-HAO CHEN^{1,2}, YAN-FENG HAN¹, ZONG-QI LIANG^{1,*} & XIAO ZOU¹

¹*Institute of Fungal Resource, College of Life Sciences &*

²*Institute of Entomology, College of Agriculture*

Guizhou University, Guiyang, Guizhou 550025, China

* CORRESPONDENCE TO: zqliang2@hotmail.com

ABSTRACT — *Gibellula clavispora*, a new fungus collected in Changbai Mountain of Jilin Province, China, is illustrated and described based on morphological traits, the DELTA (DEscription Language for TAXonomy) system, and molecular analysis. *Gibellula clavispora* is characterized by cylindrical synnemata, aspergillate conidiophores with an apical vesicle, obovoid metulae (8.6–10.8 × 2.2 µm), clavate phialides (5.4–6.5 × 1.1–2.2 µm), distinctly clavate conidia, and the absence of *Granulomanus*-synanamorphic conidiogenous cells.

KEY WORDS — araneogenous fungus, numerical classification, phenotypic traits, phenetic tree, Splitstree

Introduction

Cavara (1894:) introduced the genus *Gibellula* with *G. pulchra* Cavara as the type species. *Gibellula* species are highly specialized and apparently obligate parasites of spiders with a worldwide distribution (Samson & Evans 1992). Since the genus was established, several more species have been described on spiders (Petch 1932; Mains 1950; Samson & Evans 1973, 1977; Humber & Rombach 1987). When Samson & Evans (1992) described four new *Gibellula* species from infected spiders collected in South America, they thoroughly discussed the taxonomy, host specificity, habitats, and teleomorph connections and presented a key to the nine accepted *Gibellula* species. Tzean et al. (1997, 1998), who conducted an extensive study of the insect and spider pathogens from Taiwan since 1989, described three new *Gibellula* taxa, including *G. clavulifera* var. *major*, *G. unica*, and *G. dimorpha*. Although

more than 30 names have been published, most are treated as synonyms (<http://www.indexfungorum.org/Names/Names.asp>).

After a survey of the synnematosus entomogenous fungi in China, we reported 15 species of *Gibellula* (Chen et al. 2014), using morphological characters as the main identification criteria in the absence of any significant body of gene sequences in GenBank or other genomic databases. In order to make the taxonomy of *Gibellula* more effective and rational, additional taxonomic approaches were required for identifying a new species in *Gibellula*.

DELTA (DEscription Language for TAXonomy), a computer program developed for botanists (Dallwitz 1980) to generate taxonomic output and interactive identification, is not yet widely used by mycologists. The morphology-based DELTA expert system offers many advantages over the more traditional taxonomic approaches for animals, plants, and microbes because it uses digital standardization of morphological characters to determine which are suitable for taxonomic identification (Li et al. 1993; Li 1996; Chang et al. 2000; Carney 2003; Chen & Chen 2008; Han et al. 2009, 2010) and international communication (Chen et al. 2000a,b). We have been successful in classifying *Gibellula* species using the DELTA system (Chen et al. 2014).

In this paper, we describe and illustrate a new *Gibellula* species, *Gibellula clavispora*, which we identified by combining a classical morphological approach with the DELTA system and ITS sequence analysis.

Materials & methods

Collection and morphological examination

A *Gibellula* specimen (GZUIF07815) was obtained from a naturally infected spider collected on Changbai Mountain, Jilin Province, China, in July 2007. The specimen was oven dried at 40°C to prevent growth of fungal contaminants. Snippets of outer layer tissue from the delaminated synnemata in the dried specimen were mounted in lactophenol cotton blue solution and examined morphologically under a light microscope following procedures set forth by Liang et al. (2005). The fungus is deposited in the Institute of Fungal Resource, Guizhou University, Guiyang, China (GZUIF).

DNA extraction, amplification, and sequencing

DNA was extracted from the specimen according to Tigano-Milani et al. (1995) and stored at -20°C. TAQ enzyme and dNTP were obtained from Shanghai Sangon. The internal transcribed spacer region (ITS1+5.8S rDNA+ITS2) was amplified by polymerase chain reaction (PCR) using the primers ITS5 (5'-GGTGAGAGATTCTGTGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Han et al. 2013). After initial denaturation at 94°C for 5 min, the DNA was amplified for 35 cycles with denaturation at 94°C for 40 s, annealing at 49°C for 40 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The PCR products were purified using the UNIQ-10 column PCR product purification kit (no. SK1141; Sangon Biotech Co., Shanghai, China) according

TABLE 1. Specimens and ITS sequences used in the phylogenetic analysis
(New sequences are in bold font.)

TAXON	SPECIMEN/STRAIN	COUNTRY	GENBANK
<i>Isaria farinosa</i> (Holmsk.) Fr.	CBS 262.58	Thailand	AY624179
<i>Gibellula clavispora</i>	GZUIF07815	China	KJ857270
<i>G. clavulifera</i> var. <i>clavulifera</i>	HN0801	China	KP685596
<i>G. leiopus</i>	GZUIFR-XS0805	China	KP685597
<i>G. pulchra</i>	XCH0827	China	KP685595
<i>Gibellula</i> sp.	BCC14505	Thailand	GQ250021
<i>Gibellula</i> sp.	NHJ13158	USA	JN049864
<i>Gibellula</i> sp.	BCC02757	Thailand	GQ250020
<i>Gibellula</i> sp.	BCMU GP01	Japan	AB237661
<i>Gibellula</i> sp.	MY2566	Thailand	HM161738
<i>Torrubiella arachnophila</i>	NHJ14150	Thailand	HM161739
<i>T. arachnophila</i>	EPF083	Thailand	JX192719
<i>T. flava</i>	NBRC 30612	Japan	AB100609
<i>T. ratticaudata</i>	ARSEF 1915	USA	JN049837

to the manufacturer's protocol and sequenced with ITS5 and ITS4 at Sangon Biotech Co. The full ITS sequence (GZUIF07815) was deposited as GenBank KJ857270.

Sequence alignment and phylogenetic analyses

Sequence data from the holotype specimen of the new species were compared with ITS sequences obtained from GenBank. Representative sequences with similarities of >90% are included in our analysis. Full ITS sequences from selected known allies were also downloaded from GenBank (Benson et al. 2000). These sequences (TABLE 1) were aligned by the CLUSTAL W program (Thompson et al. 1997) using the default settings followed by manual refinements.

The sequences were analyzed by neighbor-joining (NJ) methods (Saitou & Nei 1987), maximum parsimony (MP), and maximum likelihood (ML) methods using MEGA ver. 5 (Tamura et al. 2011). NJ analysis used the default parameters, with distance values of samples calculated by the Kimura two-parameter model (Kimura 1980). ML analysis followed the GTR+G+I model (six general time reversible substitution rates, assuming gamma distributed with invariant sites). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar 2000) with search level 1, in which the initial trees were obtained with the random addition of sequences (ten replicates). Bootstrap values of the three methods were obtained from 1000 replications per analysis. Other parameters followed the default conditions. *Isaria farinosa* (Holmsk.) Fr. was designated as outgroup.

A network was created by Splitstree version 4.10 (Huson & Bryant 2006) using the sequences from the phylogenetic tree except for the outgroup. The running conditions were *P*-distance, Unrooted Neighbor Net.

DELTA system

Fifteen *Gibellula* species were used in the study (TABLE 2). A data matrix (TABLE 3) was generated using published character data from species descriptions (Chen et al. 2014). These characters were then used to generate a phenetic tree (FIG. 3; Chen et al. 2014) in DELTA using the PCLASS program in accordance with the program CONFOR using the Editor “todis” directives.

TABLE 2. *Gibellula* spp. used for constructing the DELTA database

TAXON
<i>G. alata</i> Petch
<i>G. brunnea</i> Samson & H.C. Evans
<i>G. clavata</i> Samson & H.C. Evans
<i>G. clavispora</i>
<i>G. clavulifera</i> var. <i>alba</i> Humber & Rombach
<i>G. clavulifera</i> (Petch) Samson & H.C. Evans var. <i>clavulifera</i>
<i>G. clavulifera</i> var. <i>major</i> Tzean et al.
<i>G. curvispora</i> Z.Q. Liang et al.
<i>G. dabieshanensis</i> B. Huang et al.
<i>G. dimorpha</i> Tzean et al.
<i>G. leiopus</i> (Vuill. ex Maubl.) Mains
<i>G. mainsii</i> Samson & H.C. Evans
<i>G. mirabilis</i> Samson & H.C. Evans
<i>G. pulchra</i> Cavara
<i>G. unica</i> L.S. Hsieh et al.

TABLE 3. Phenetic characters and their states for species of *Gibellula*.

1. Host	a. spider; b. insect
2. Synnemata	a. present; b. absent
3. Synnema state	a. solitary; b. in pairs; c. scattered; d. clustered
4. Synnema shape	a. clavate; b. cylindrical
5. Synnema tip	a. bulbous; b. acuminate
6. Conidiophores	a. penicillate; b. aspergillate
7. Conidiophore surface	a. smooth; b. rough
8. Conidiophores bases	a. pigmented; b. hyaline
9. Conidiophore length	(µm)
10. Vesicle	a. present; b. absent or hardly developed
11. Metulae shape	a. ovoid; b. ellipsoidal; c. obovoid; d. clavate; e. cylindrical
12. Phialide shape	a. clavate; b. cylindrical; c. ellipsoidal
13. Phialide neck	a. present; b. absent
14. Phialide length	(µm)
15. Phialide width	(µm)
16. Conidium shape	a. clavate; b. ovoid; c. allantoid; d. fusiform; e. ellipsoid
17. Conidium surface	a. smooth; b. apiculate
18. Conidium length	(µm)
19. Conidium width	(µm)
20. <i>Granulomanus</i> synanamorph	a. present; b. absent
21. <i>Granulomanus</i> conidiophores	a. rough; b. smooth

Results

Phylogenetic analysis

For MP analysis of the 14-taxon ITS dataset (150 positions), the consistency index was 0.892857, the retention index was 0.911756, and the composite index was 0.814076 (0.785714) for all sites (parsimony-informative sites). ML analysis of the same dataset produced a tree with a log likelihood of -349.60. The ITS-based phylogram (FIG. 1) clearly supports the new species, *Gibellula clavispora*, in an independent clade.

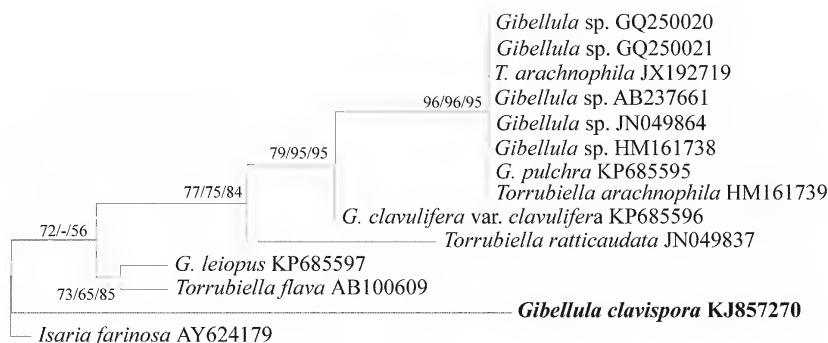


FIG. 1. Phylogenetic tree generated based on the ITS sequences. Statistical support values (≥50%) are shown at nodes, for neighbor-joining/maximum parsimony/maximum likelihood.

Taxonomy

Gibellula clavispora Z.Q. Liang, Wan H. Chen & Y.F. Han, sp. nov.

FIG. 2

MYCOBANK MB 810567

Differs from other *Gibellula* species by its cylindrical synnemata, aspergillate conidiophores, obovoid metulae, clavate phialides, and distinctly clavate conidia.

TYPE: China, Jilin Province, Changbai Mountain, spider, 15 August 2007, A.Y. Liu (Holotype, GZUIF07815).

ETYMOLOGY: referring to the shape of conidia.

HOST: a species of spider completely covered by white mycelium. SYNNEMATA solitary, arising from the host, cylindric, slender, scattered, attenuated, and composed of parallel, multiseptate longitudinal hyphae. HYPHAE branched, hyaline, smooth. CONIDIOPHORES hyaline, septate, arising from aerial mycelium or synnemata, scattered, 96–113 µm long, septa conspicuous, smooth or occasionally roughened along the length, narrowing abruptly to a slender apex, and terminating in a swollen vesicle. VESICLE obovate, hyaline, smooth-walled. PHIALIDES clavate, smooth-walled, with a short neck, hyaline,

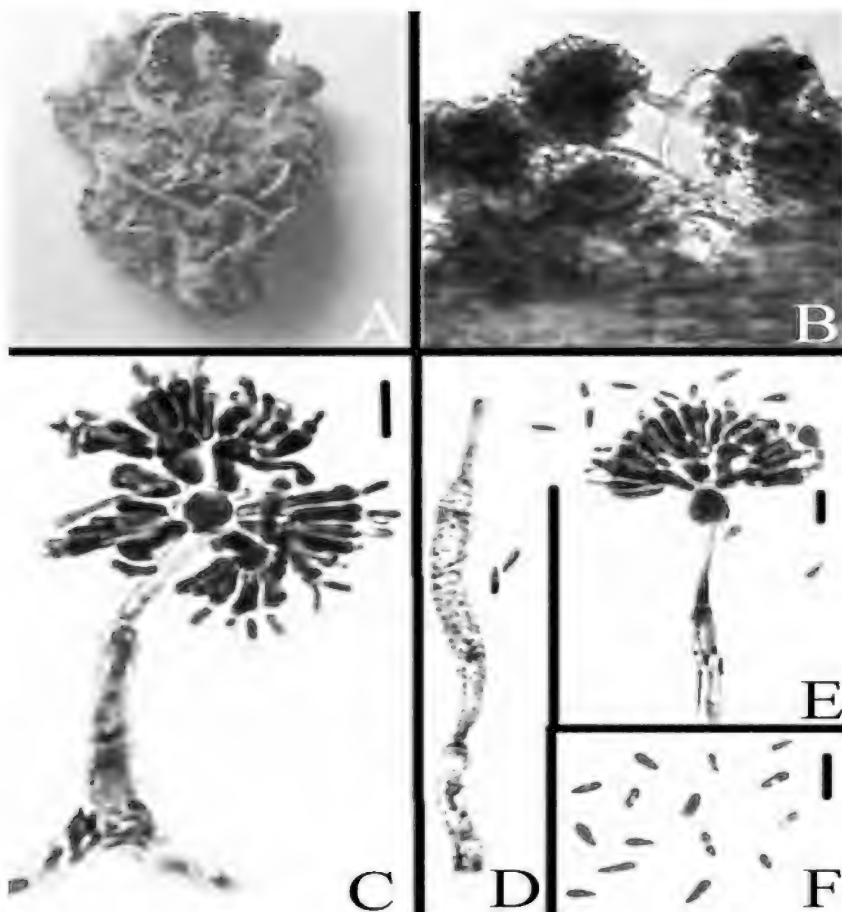


FIG. 2. *Gibellula clavispora* (holotype, GZUIF07815) A: infected spider; B: conidiophores arising from the synnema; C–E: conidiophores with smooth or occasionally rough surface; F: conidia. Scale bars: C, E, F = 10µm.

8.6–10.8 $\times$ 2.2 µm. METULAE obovate, hyaline, smooth-walled, 7.6–8.6 $\times$ 3.2 µm, borne on vesicle. CONIDIA clavate, hyaline, smooth-walled, single, 5.4–6.5 $\times$ 1.1–2.2 µm, occasionally ellipsoidal, 2.2–4.3 $\times$ 1.1 µm. Neither teleomorph nor *Granulomanus* synanamorph observed.

COMMENTS: Three other *Gibellula* species produce clavate conidia—*G. alata*, *G. brunnea*, and *G. mainsii* (TABLE 4). *Gibellula alata* is distinguished by its short slender synnemata terminating in a bulbous outgrowth from which a number of conidiophores and diagnostic wing-like structure arise. *Gibellula brunnea*

TABLE 4. Morphological comparison between *Gibellula clavispора* and allied species.

SPECIES	SYNNEMA APEX	GRANULOMANUS SYNANAMORPH	PHIALIDE SHAPE & SIZE (μm)	CONIDIAL SHAPE & SIZE (μm)
<i>G. alata</i>	bulbous	absent	clavate, 9–13 × 3–4	oblong-oval or clavate, 4–9 × 2–4
<i>G. brunnea</i>	acuminate	present	cylindrical, 10–13 × 3–4	fusiform or sl. clavate, 8–10 × 2–2.5
<i>G. clavispора</i>	acuminate	absent	clavate, 8.6–10.8 × 2.2	clavate, 5.4–6.5 × 1.1–2.2
<i>G. mainsii</i>	acuminate	absent	cylindrical, 10–13 × 3–4	fusiform or sl. clavate, 8–10 × 2–2.5

differs from *G. clavispора* in its *Granulomanus* synanamorph state, while *G. mainsii* differs in its cylindrical phialides.

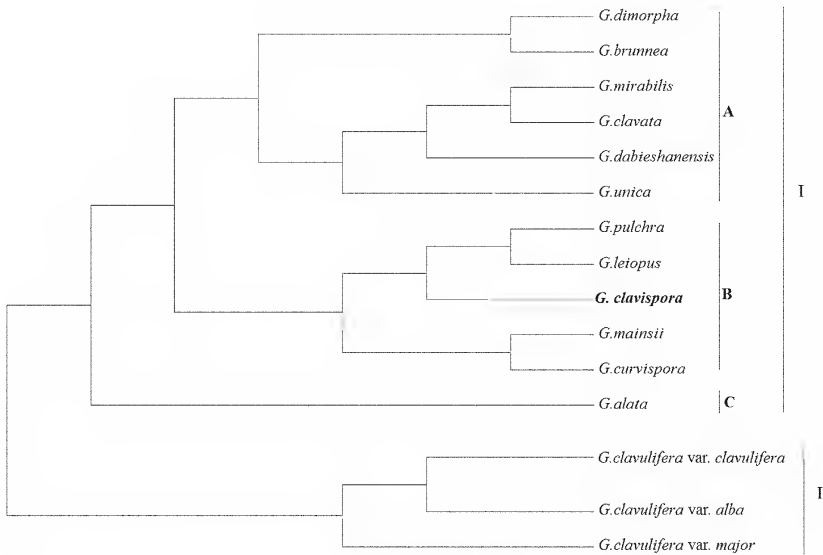


FIG. 3. Phenetic tree generated for *Gibellula* spp. by DELTA.

DELTA system analysis

DELTA system analysis is a numerical character classification. It was not granted special priority, so that all characters selected were weighted equally in this study. The phenetic tree (FIG. 3) based on the morphological characteristics presented in TABLE 3, separates *Gibellula* into several distinct groups. The new species *G. clavispора* was grouped together with *G. pulchra*, *G. leiopus*,

G. mainsii, and *G. curvispora* on a branch nested within the other species of this genus.

Discussion

The solitary septate conidiophores of this new species are scattered on the host body or synnemata, usually with small but distinct terminal vesicle bearing one or two orders of the tightly clustered metulae characteristic of *Gibellula* species (Humber & Rombach 1987). *Gibellula* is further distinguished from allied genera by its spider-specific species characterized by septate conidiophores that narrow abruptly to a slender apical neck (Samson et al. 1992). It is obvious that *G. clavispora* belongs in *Gibellula* and represents a new species morphologically distinguished by cylindrical synnemata, aspergillate conidiophores, obovoid metulae, clavate phialides, distinctly clavate conidia, and the absence of conidiogenous cells of *Granulomanus* synanamorph.

The DELTA system analysis produced a clustering diagram dividing *Gibellula* into two distinct groups (I–II) and three subgroups (A–C). The two groups are separated by aspergillate (Group I) and penicillate (Group II) conidiophores, which is consistent with the findings of Tzean et al. (1997).

Group II taxa—*G. clavulifera* var. *clavulifera*, *G. clavulifera* var. *alba*, *G. clavulifera* var. *major*—are characterized by solitary and attenuated synnemata, penicillate conidiophores, and the absence of vesicles.

Group I is divided into three subgroups. Subgroup A, comprising *G. dimorpha*, *G. mirabilis*, *G. clavata*, *G. dabieshanensis*, and *G. unica*, is characterized by solitary or paired, attenuated synnemata, aspergillate conidiophores, cylindrical phialides, and fusiform conidia. Subgroup B (*G. pulchra*, *G. leiopus*, *G. clavispora*, *G. mainsii*, *G. curvispora*, and *G. brunnea*) is characterized by cylindrical and scattered synnemata and the absence of a *Granulomanus* synanamorph. Subgroup C, containing only *G. alata*, is distinguished by the flattened-globose head and wing-like extension at the terminal apex of its synnemata.

DELTA system analysis revealed that some characters, such as conidiophore shape and the presence/absence of vesicle and *Granulomanus* synanamorph, could help us conveniently identify species. The phenetic tree places the new species *G. clavispora* on an independent branch, as separated by its clavate conidia, aspergillate conidiophores, and absence of *Granulomanus* synanamorph.

The ITS-derived phylogram (Fig.1) also placed *G. clavispora* in an independent clade. The phylogenetic and phenetic trees were not congruent, possibly due to inherent differences between morphological and molecular characters.

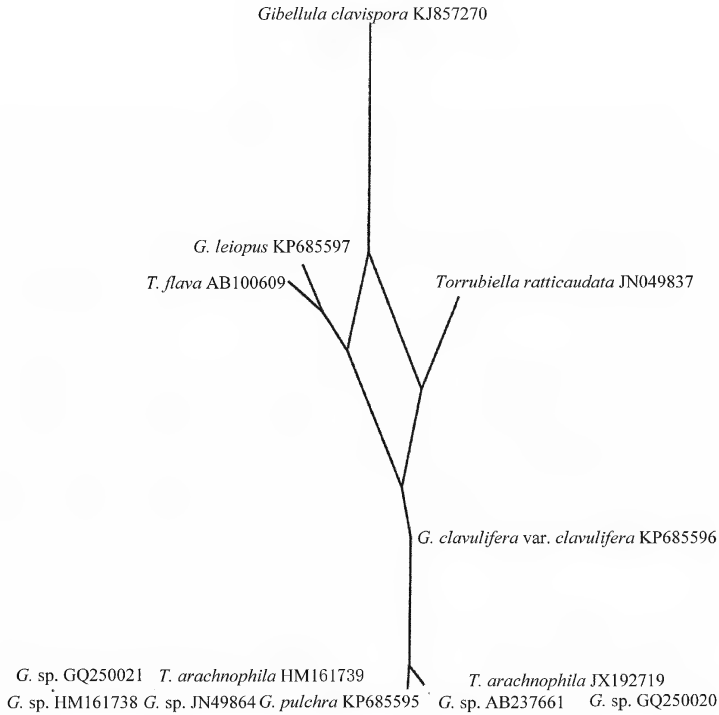


FIG. 4. Reconstruction of the Neighbor-Net network from taxa in FIG. 1.

We investigated the incongruence in the horizontal processes using SplitsTree4 (Huson & Bryant 2006), which produced a recomputed neighbor-net (FIG.4) with a 99.999 fit and 99.999 LS fit. The result revealed an ambiguous signal present between *G. clavispora* and other species, with *G. clavispora* on a separate branch in the neighbor-net. Combining morphology and phylogenetic tree, Neighbor-Net analyses support *Gibellula clavispora* as a new taxon in *Gibellula*.

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The authors are grateful for pre-submission comments and suggestions provided by Prof. Richard A. Humber & Prof. Bo Huang. We thank Prof. A.Y. Liu for collecting the specimen and providing the ecological record about this fungus. We also warmly thank Dr. S.R. Pennycook and Dr. L.L. Norvell for editorial review and revisions. The research is supported by Major Program of National Natural Science Foundation of China (31093440, 31493010 & 31493011), the National Basic Research Priorities Program of China (2013FY110400) and Youth Foundation of Guizhou Province (No. 2013-05).

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***Olpitrichum sphaerosporum*: a new USA record and phylogenetic placement**

DE-WEI LI^{1, 2}, NEIL P. SCHULTES^{3*} & CHARLES VOSSBRINCK⁴

¹*The Connecticut Agricultural Experiment Station, Valley Laboratory,
153 Cook Hill Road, Windsor, CT 06095*

²*Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University,
Nanjing, Jiangsu 210037, China*

³*The Connecticut Agricultural Experiment Station, Department of Plant Pathology and Ecology,
123 Huntington Street, New Haven, CT 06511-2016*

⁴*The Connecticut Agricultural Experiment Station, Department of Environmental Sciences,
123 Huntington Street, New Haven, CT 06511-2016*

* CORRESPONDENCE TO: neil.schultes@ct.gov

ABSTRACT — *Olpitrichum sphaerosporum*, a dimorphic hyphomycete isolated from the foliage of *Juniperus chinensis*, constitutes the first report of this species in the United States. Phylogenetic analyses using large subunit rRNA (LSU) and internal transcribed spacer (ITS) sequence data support *O. sphaerosporum* within the *Ceratostomataceae*, *Melanosporales*.

KEY WORDS — asexual fungi, *Chlamydomyces*, *Harzia*, *Melanospora*

Introduction

Olpitrichum G.F. Atk. was erected by Atkinson (1894) and is typified by *Olpitrichum carpophilum* G.F. Atk. Five additional species have been described: *O. africanum* (Saccas) D.C. Li & T.Y. Zhang, *O. macrosporum* (Farl. Ex Sacc.) Sumst., *O. patulum* (Sacc. & Berl.) Hol.-Jech., *O. sphaerosporum*, and *O. tenellum* (Berk. & M.A. Curtis) Hol.-Jech. This genus is dimorphic, with a *Proteophiala* (*Aspergillus*-like) synanamorph.

Chlamydomyces Bainier and *Harzia* Costantin are dimorphic fungi also with a *Proteophiala* synanamorph (Gams et al. 2009). *Melanospora* anamorphs comprise a wide range of genera including *Acremonium*, *Chlamydomyces*,

TABLE 1. ITS and LSU sequences of *Olpitrichum sphaerosporum* and allied species used in this study

TAXON	ORIGIN	SUBSTRATE	VOUCHER No.	GENBANK No.		REFERENCE
				ITS	LSU	
<i>Olpitrichum sphaerosporum</i>	USA	Foliage of <i>Juniperus chinensis</i>	UAMH 11865	KT221061*	KT221062*	This paper
<i>Bertia tropicalis</i>	Panama	Branch	SMH3513	—	AY695263	Huhndorf et al. (2004)
<i>Chaetosphaerella phaeostroma</i>	Costa Rica	—	SMH4257	—	AY695264	Huhndorf et al. (2004)
<i>Coriolospora maritima</i>	—	—	PP1991	—	AF491260	Campbell et al. (2002)
<i>Harzia actenionoides</i>	USA	Seed	NRRL 54328	HQ288051	—	Wicklow DT; unpublished
<i>H. camerounensis</i>	USA	Seed of <i>Zea mays</i>	NRRL 54327	HQ698593	—	Wicklow DT; unpublished
<i>Hirsutella stihelliformis</i> var. <i>myrmicarum</i>	Cameroon	Unknown creeper plant	CBS 136420 [T]	KF777163	KF777216	Crous et al. (2013)
	UK	<i>Myrmica rubra</i>	IMI 396398 [T]	—	GQ866967	Evans et al. (2010)
<i>Melanospora brevirostris</i>	Canada	Alpine regosol	ATCC42427	—	AY015627	Zhang & Blackwell (2002)
<i>M. singaporensis</i>	Singapore	Soil	ATCC 38286 [T]	—	AY015629	Zhang & Blackwell (2002)
<i>M. pascuensis</i>	Easter I., Chile	Soil	IMI 378527 [T]	AJ011312	—	Stchigel et al. (1999)
<i>M. tiffanyae</i>	Canada	—	SMCD2222	FJ748921	FJ748915	Vujanovic V; unpublished
	USA	Contaminant	ATCC15515 [T]	—	AY015630	Zhang & Blackwell (2002)
<i>M. zamiae</i>	Indonesia	Air	ATCC 12340	—	AY046579	—
<i>Mitroscus trigonosporus</i>	—	—	RSA 1942	—	U47835	Spatafora J; unpublished
<i>Papulaspora sepedonioides</i>	Canada	Fallen female cones of <i>Picea glauca</i>	UAMH 10707	—	EU518666	Davey et al. (2008)
<i>Petriella setifera</i>	Hong Kong	Wood panel in coastal water	CBS 437.75	—	DQ470969	Zhang et al. (2006)
<i>Scortechinia conferta</i>	USA	—	SMH2648	—	AY695272	Huhndorf et al. (2004)
<i>Sphaerodes compressa</i>	Nepal	Soil, cow dung	IMI212200	—	AY015633	Zhang & Blackwell (2002)
<i>S. fimicola</i>	USA	Sclerotium of <i>Aspergillus flavus</i>	NRRL 54804	JQ034510	—	Wicklow DT; unpublished
<i>S. pseudofimicola</i>	Japan	Hare dung	ATCC26180	—	AY015628	Zhang & Blackwell (2002)
<i>S. quadrangularis</i>	Australia	Forest soil	CBS 108937	JN709487	—	Li & Wang; unpublished
<i>Tolypocladium capitatum</i>	Spain	Foil	CBS 112763 [T]	—	GQ354530	Garcia et al. (2004)
<i>Vittatispora coorgii</i>	—	<i>Elaphomyces</i> sp.	OSC71233	—	AY489721	Castlebury et al. (2004)
<i>Xylaria hypoxylon</i> ¹	India	Foil	BICC 7817 [T]	—	DQ017375	Chaudhary et al. (2006)
	USA	Wood	ATCC 42768	AY327477	U47841	Platas et al. (2004)

* new sequences; — information not available; [T] ex-type; ¹ outgroup.

Harzia, *Papulaspora*, *Paecilomyces*, *Phialophora*, and *Proteophiala* (Ciferri 1958, Kendrick & DiCosmo 1979, Cannon & Hawksworth 1982, Rehner & Samuels 1995, Hawksworth et al. 1999, Davey et al. 2008, Kirk et al. 2008, Gams et al. 2009). Some *Papulaspora* species reportedly also have a *Proteophiala* synanamorph (Gams & Domsch 1969, Domsch et al. 1980, Seifert et al. 2011). The phylogenetic relationship among genera that have a *Proteophiala* synanamorphic state remains uncertain.

No teleomorph for *Olpitrichum* has yet been reported, and a search of GenBank showed no DNA sequence data for *Olpitrichum*. Here we present the LSU and ITS DNA sequences for *Olpitrichum sphaerosporum*. Among the genera sharing the *Proteophiala* synanamorph, we found no DNA sequence data for *Chlamydomyces*, two ITS sequences for *Harzia acremonioides*, one ITS and one LSU for *H. cameroonensis*, and one LSU for *Papulaspora sepedonioides*. The systematic assignment of *Olpitrichum* is ambiguous, except that the members of this genus belong to the asexual fungi of *Pezizomycotina*, *Ascomycota* (Index Fungorum 2015).

Several studies have been conducted to elucidate the phylogenetic relationships of *Melanospora* and its allies (Rehner & Samuels 1995, Zhang & Blackwell 2002, Zhang et al. 2006, Hibbett et al. 2007). These studies having indicated *Melanospora* as a sister taxon to the *Ceratostomataceae* (Zhang & Blackwell 2002, Hibbett et al. 2007), the new order *Melanosporales* was proposed in Hibbett et al. (2007) to accommodate *Melanospora* and its allies. Phylogenetic analysis using LSU sequence data indicated that *Papulaspora sepedonioides* Preuss also belongs to *Melanosporales* (Davey et al. 2008).

Olpitrichum sphaerosporum, isolated from the foliage of *Juniperus chinensis* in Windsor, Connecticut, constitutes the first record of this fungus in the United States. Here we shed new light on the placement of *O. sphaerosporum* based upon ITS and LSU DNA sequence analyses.

Materials & methods

A sample of *Juniperus chinensis* with a purported foliar disease was submitted to the Inquiry Office of The Connecticut Agricultural Experiment Station Valley Laboratory for diagnosis in February 2014. The sample was placed in a plastic bag with a piece of wet paper towel for three days at room temperature. Following incubation, a fungus grew on the necrotic part of the foliage. A sterile needle was used to transfer the fungal spores to a 2% malt extract agar (MEA) plate. Fungal isolates were grown on MEA at 25°C for 7 days. Conidiophores and conidia were mounted in 85% lactic acid. All microscopic observations were made under an Olympus BX40 microscope with Nomarski differential interference contrast optics. Photomicrographs were taken with an Olympus Microfire digital camera (Goleta, CA). Measurements of the fungal structures were statistically analyzed for means and standard deviations with 95% confidence interval of means.

DNA was extracted from a petri plate-grown colony according to the procedure in ZR Fungal/Bacterial DNA MicroPrep Kit (Zymo Research, Irvine, CA, USA). Oligonucleotides ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used with isolated genomic DNA to amplify a fragment corresponding to the small subunit rDNA internal transcribed spacer (ITS) region by polymerase chain reaction (PCR) (Gardes & Bruns 1993). Oligonucleotides 5.8SR (5'-TCGATGAAGAACGCAGCG-3') and LR7 (5'-TACTACCACCAAGATCT-3') were used to amplify the ITS region and a portion of the large subunit rDNA region by PCR (Gargas & DePriest 1996). The resulting PCR products were purified using QIA quick PCR Purification columns (Qiagen, Valencia, CA, USA). The ITS PCR product was sequenced using oligonucleotides ITS1, ITS4, ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'), and ITS3 (5'-GCATCGATGAAGAACGCAGC-3'). The LSU PCR product was sequenced using primers LROR (5'-ACCCGCTGAAGCTTAAGC-3'), LR7 (5'-TACTACCACCAAGATCT-3'), LR5 (5'-TCCTGAGGGAACCTTCG-3'), LR3R (5'-GTCTTGAAACACGGACC-3'), and LR16 (5'-TTCCACCCAAACACTCG-3'). DNA sequence information was deposited to GenBank as accession KT221061 for the ITS region and KT221062 for the LSU region.

Sequence similarity searches and comparisons were conducted using MegaBLAST (Wheeler et al. 2003) against the NCBI nucleotide database. *Harzia*, *Olpitrichum*, and several additional allied genera were chosen for phylogenetic analysis (TABLE 1). *Xylaria hypoxylon* (ATCC 42768) was designated as outgroup.

Sequences were aligned using ClustalW (Thompson et al. 1994). Phylogenetic analyses were conducted using the neighbor joining and maximum likelihood procedures in MEGA6 (Tamura et al. 2013). A bootstrap test was carried out with 1000 replicates.

Results

Taxonomy

Olpitrichum sphaerosporum Matsush., Icon. Microfung. Matsush. Lect.: 103 (1975)

FIG. 1

COLONIES on MEA effuse, cottony, pale ochraceous to yellow-buff, attaining 100 mm in diam. at 25°C in 7 days; aerial hyphae abundant; some mycelia reaching the lid of the plate in 7 days. CONIDIOPHORES erect, simple or sparsely branched at right angles, (100–)150–400(–500) µm long, 4–6(–7) µm wide at the base and the middle, 4–6 septate, thick-walled, smooth. Terminal fertile part of conidiophores 5–15 µm long, often extending, with 3–6 denticle conidiogenous cells. Conidiogenous loci denticles arranged in irregular verticils, tapering to the apex, 4–15 µm long, 7–9 µm wide at the base, rarely constricted at the base, often branched and developing new denticles or extending and forming new conidiophores. CONIDIA unicellular, solitary, subhyaline to pale ochraceous, spherical or subspherical, smooth or verruculose, sometimes with a small papilla at the base, (18–)19–23(–25) × (15–)18–22(–25) µm (n = 30), with wall 0.5–1 µm thick. Conidial secession rhexolytic.

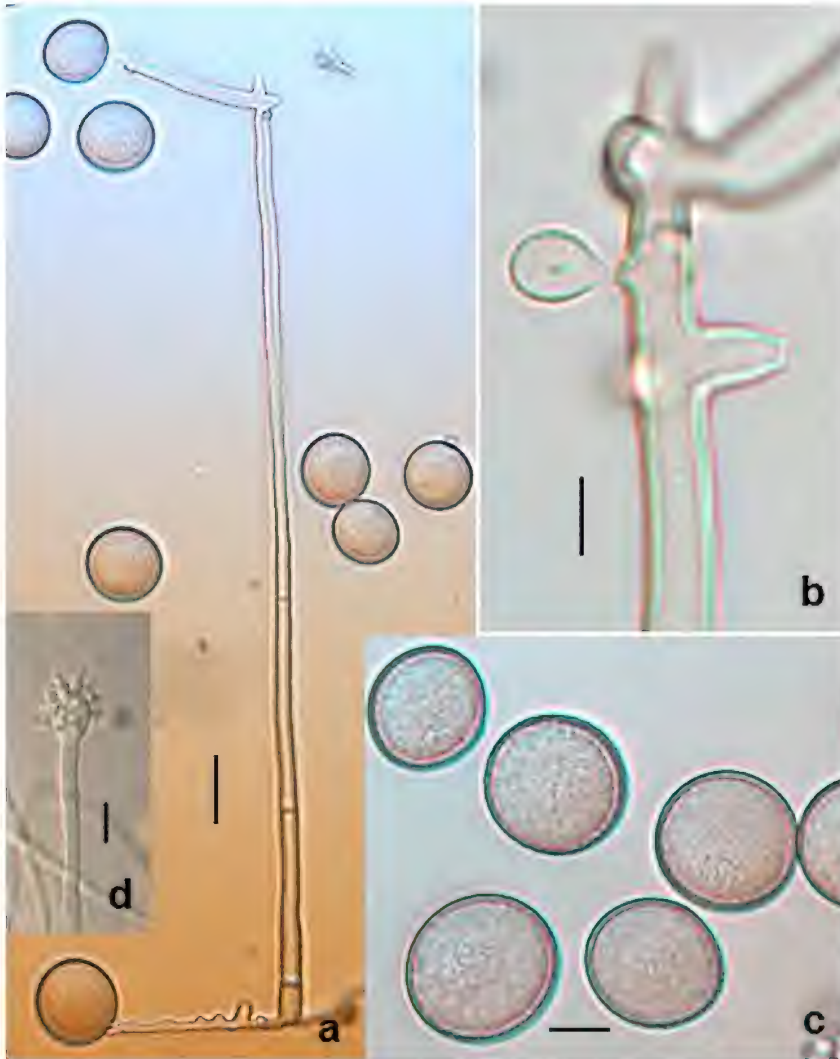


FIG. 1. *Olpitrichum sphaerosporum* (UAMH 11865) a. Conidiophore and conidia; b. Conidiogenous cells and an immature conidium; c. Conidia; d. *Proteophiala* synanamorph. Scale bars: a = 20 µm; b = 5 µm; c, d = 10 µm.

Synanamorph *Proteophiala* (*Aspergillus*-like), scattered in basal hyphae; conidiophores colorless, smooth, phialides $6\text{--}7.5 \times 3\text{--}4$ µm, conidia catenate, obovoid with a truncate base, colorless, smooth, $2\text{--}4 \times 1.5\text{--}3$ µm in diam.

MATERIAL EXAMINED: USA, CONNECTICUT, Windsor, 41°51'04"N 72°39'39"W, dead foliage of *Juniperus chinensis* L. (*Cupressaceae*), Feb 24, 2014, coll. De-Wei Li, UAMH 11865 (DWL2014-6).

HOSTS/SUBSTRATES: saprobe, on dead foliage of *Juniperus chinensis*, dead pericarp of *Camellia japonica* L., decaying leaf of palm.

DISTRIBUTION: Japan (Matsushima 1975), Mexico (Heredia Abarca & Arias Mota 2008), USA.

NOTE: *Olpitrichum sphaerosporum* represents a new record for the USA.

Phylogenetic analyses

Maximum likelihood and neighbor joining analyses produced the same results regarding the relationships within the *Melanosporales*. LSU sequence analyses indicated that *Olpitrichum sphaerosporum* and members of the *Ceratostomataceae*, *Melanosporales*—*Harzia*, *Melanospora*, *Papulaspora*, *Sphaerodes*, *Vittatispora*—form a monophyletic lineage (FIG. 2), clearly

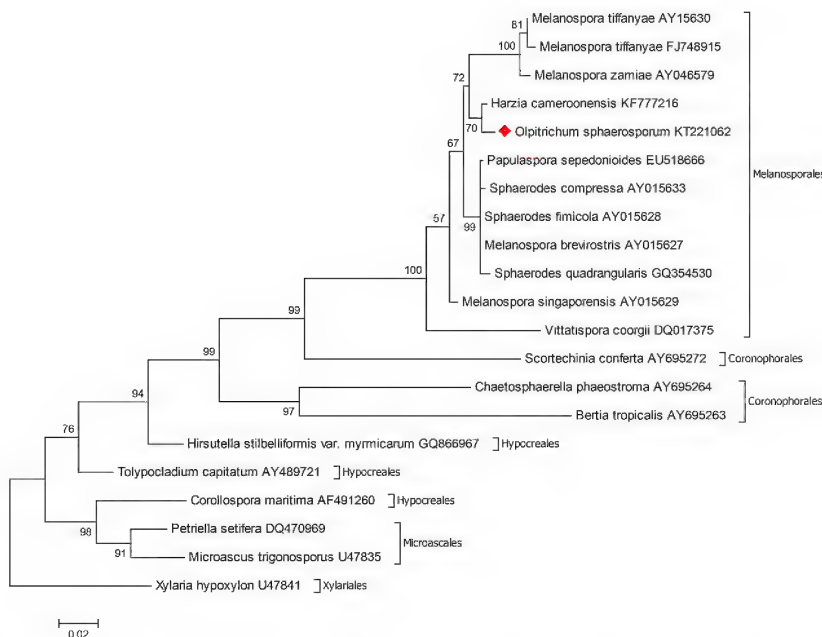


FIG. 2. LSU sequence maximum likelihood analysis of *Olpitrichum sphaerosporum* and related species (1000 bootstrap replicates; bs values indicated). Species names precede sequence accession numbers. *Xylaria hypoxylon* U47841 represents the outgroup. The scale bar indicates the number of expected changes per site.

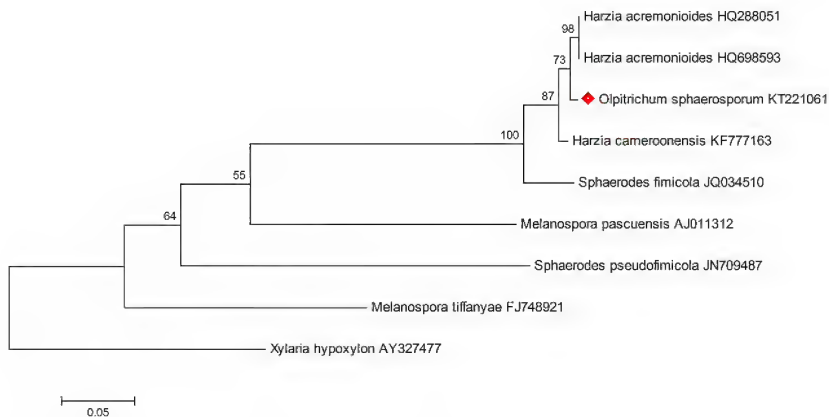


FIG. 3. ITS sequence maximum likelihood analysis of *Olpitrichum sphaerosporum* and allied fungi (1000 bootstrap replicates; bs values indicated). Species names precede sequence accession numbers. *Xylaria hypoxylon* AY327477 represents the outgroup. The scale bar indicates the number of expected changes per site.

supporting *Olpitrichum sphaerosporum* within the *Ceratostomataceae*. ITS sequence analyses resolved the phylogenetic relationship between *Olpitrichum sphaerosporum* and *Harzia* species more finely and grouped them in the same clade, indicating that *O. sphaerosporum* and *Harzia* (FIG. 3) are congeneric.

Discussion

This is the first report of *Olpitrichum sphaerosporum* from the United States. Among the six *Olpitrichum* species, only *O. sphaerosporum* develops spherical conidia. The Connecticut isolate differs slightly from the Japanese type isolate (Matsushima 1975) in having longer conidiophores.

The Connecticut isolate grows very slowly on DG18 media (less than 2 mm at 25°C in 7 days). It completely lost viability when stored in water at 4°C for 6 months, and only a few conidia survived when cryopreserved at -70°C for a year. This poses a challenge for culture maintenance.

Olpitrichum is morphologically similar to *Chlamydomyces* and *Harzia* (Holubová-Jechová 1974, Seifert et al. 2011) and all display a *Proteophiala* synanamorph (Seifert et al. 2011). *Chlamydomyces* and *Harzia* are both connected to the same sexual genus, *Melanospora* (Rehner & Samuels 1995).

As noted above, no rDNA sequence information for *Olpitrichum* and *Chlamydomyces* was available prior to this report. Both our phylogenetic

analyses (FIGS 2, 3) and the morphologies of the asexual and sexual states suggest that phylogenetically *Olpitrichum*, *Harzia*, *Melanospora*, and *Sphaerodes* are closely related.

Harzia Costantin was erected by Costantin (1888) and typified by *H. acremonioides* (Harz) Costantin. *Harzia acremonioides*, *H. cameroonensis*, and *Olpitrichum sphaerosporum* were included in the phylogenetic ITS sequence analysis. As our results reveal that *Olpitrichum* and *Harzia* are closely related (FIG. 3), we conclude that either *Harzia* should remain a paraphyletic genus or that *Olpitrichum sphaerosporum* should be transferred to *Harzia*. With no culture of the type species, *Olpitrichum carpophilum*, currently available, it is premature to merge the two genera until the type specimen or verifiable material from the type species is available for DNA sequencing.

Previous studies have placed *Harzia*, *Melanospora*, *Sphaerodes*, *Vittatispora*, and *Papulaspora sepedonioides* within *Ceratostomataceae*, *Melanosporales* (Zhang & Blackwell 2002, Chaudhary et al. 2006, Davey et al. 2008, Lackner et al. 2014, Index Fungorum 2015), and Seifert et al. (2011) speculated that *Olpitrichum* might belong to *Ceratostomataceae*, *Melanosporales* according to its morphology. Our LSU sequence analysis groups *Olpitrichum sphaerosporum* in the same clade as the aforementioned genera (FIG. 2) and clear molecular evidence supports *Olpitrichum sphaerosporum* within the *Ceratostomataceae*, *Melanosporales*.

The studies of Zhang & Blackwell (2002) and Chaudhary et al. (2006) grouped *Sphaerodes* and *Melanospora* in the same clade, and Chaudhary et al. (2006) suggested that *Sphaerodes* and *Melanospora* are congeneric based on their phylogenetic analysis. Neither study included anamorphic genera, such as *Olpitrichum* and *Harzia*. Since the anamorph of *Sphaerodes* remains unknown and no DNA sequence data are available for the type species, *Sphaerodes episphaerium* (W. Phillips & Plowr.) Clem., the true phylogenetic relationships of *Sphaerodes* with *Melanospora* and other closely related asexual genera (*Olpitrichum* and *Harzia*) remain to be determined.

Olpitrichum, *Chlamydomyces*, and *Harzia* share the same *Proteophiala* synanamorph. However, the phylogenetic relationship of *Chlamydomyces* with *Olpitrichum* and *Harzia* remains unknown. From our results we could not determine the relationships among *Melanospora*, *Sphaerodes*, and *Papulaspora*. *Papulaspora* is a phylogenetically heterogeneous genus, and its members have been linked to numerous teleomorphs including *Ascobolus*, *Chaetomium*, *Cubonia*, *Hypomyces*, *Microthecium*, *Lophium*, *Melanospora*, *Neurospora*, and *Sordaria* (Seifert et al. 2011). *Papulaspora sepedonioides* is the type species of the genus. Thus, *Papulaspora* taxa not phylogenetically related to *Melanospora* should be excluded from *Papulaspora* and moved to other taxonomic groups.

However, as *Melanospora* is apparently also polyphyletic, further studies on these genera are necessary.

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***Sarcogyne saphyniana* sp. nov., a saxicolous lichen from northwestern China**

LAZZAT NURTAI¹, KERRY KNUDSEN² & ABDULLA ABBAS^{1*}

¹College of Life Science, Arid Land Lichen Research Center of Western China,
Xinjiang University, Urumqi, 830046, P. R. China

²Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences,
Prague, Kamýcká 129, Praha 6 - Suchbátka, CZ-165 21, Czech Republic

* CORRESPONDENCE TO: zxg_lichen@163.com

ABSTRACT — A new species of *Sarcogyne* in the *badiofusca* group is described from northwest China. It differs from *Acarospora badiofusca* in having an algal layer interrupted by wide hyphal bands.

KEY WORDS — *Acarosporaceae*, diversity, phylogeny, taxonomy

Introduction

The crustose lichen genus *Sarcogyne* Flot. (*Acarosporaceae*) is characterized by black or red lecideine apothecia with or without a carbonized exciple, a non-carbonized epihymenium, a hymenium with or without branching paraphyses, and asci containing 50–100 or more simple hyaline ascospores (Knudsen & Standley 2007, Fletcher & Hawksworth 2009). The thallus may be endolithic or epilithic. Species occur on non-calcareous or calcareous rock, on soil in biological soil crusts, and one species (*S. lapponica* (Ach.) K. Knudsen & Kocourková) is known only from a historic collection on wood (Knudsen & Kocourková 2008). The genus contains approximately 40 species, by our estimate. However, it contains many more names than species, because the monographer Magnusson included in *Sarcogyne* many species with a carbonized epihymenium later transferred to *Polysporina* Vězda, but which actually belong in *Acarospora* A. Massal. where they have yet to be transferred (Magnusson 1935 a, & b; Westberg et al. 2015). Recent phylogenetic studies have shown that many current *Acarospora* species should be referred to *Sarcogyne*,

including such well-known species as *Acarospora glaucocarpa* (Ach.) Körb., *A. cervina* (Ach.) A. Massal., and *A. badiofusca* (Nyl.) Th. Fr. (Reeb et al. 2004, Miadlikowska et al. 2014, Westberg et al. 2015). However, the backbone of the most recently published phylogenetic tree is weak, suggesting that *Sarcogyne* is not monophyletic; it is expected that the genus will eventually be divided into several genera (Westberg et al. 2015). For the sake of nomenclatural stability, some current *Acarospora* species (such as *A. badiofusca*) have not yet been transferred to *Sarcogyne*, although they clearly do not belong to *Acarospora* s. str.

In this paper we describe a new *Sarcogyne* species from the mountains of Xinjiang in northwestern China that belongs to the *A. badiofusca* group, a group characterized as having algal layers that may or may not be interrupted by hyphal bundles (Knudsen et al. 2014). The only member of this group that has thus far been sequenced is *A. badiofusca*, which phylogenetic analyses place at the end of a long branch with *A. cervina* (which has an interrupted algal layer) but embedded within *Sarcogyne* (Westberg et al. 2015). Our new species, which has lecideine apothecia and a non-carbonized epihymenium, anatomically also fits the current concept of *Sarcogyne*.

Materials & methods

The examined specimens (including the holotype) are preserved in Arid Land Lichen Research Center of Western China, Xinjiang University, Urumqi, China (XJU-NALH). They were morphologically examined with Olympus STM7 microscope and photographed taken with a Nikon DS-Fi2 and Canon PC1200. Measurements were made in water and amyloid reaction was tested with Lugol's (I). The lichen was checked for secondary metabolites in Solvent C using thin layer chromatography techniques (TLC) (Culbertson, 1972).

Taxonomy

Sarcogyne saphyniana A. Abbas, Nurtai & K. Knudsen, **sp. nov.**

FIGS 1, 2

MYCOBANK MB 815828

Differs from *Acarospora badiofusca* by its algal layer divided by wide hyphal bands into palisades and clumps of algal cells.

TYPE — China, Xinjiang, Tianshan Grand Canyon, 43°18.89'N 87°19.51'E, alt. 2257 m, sandstone, 10 Jul 2014, A. Abbas, B. Memet, L. Nurtai 20140414 (Holotype, XJU-NALH).

ETYMOLOGY — *Sarcogyne saphyniana* is named in honor of Saphyn, a wise man from Tartar Republic who in 1910 came to Xinjiang, where he helped the poor people and established a school.

Thallus squamulose to areolate, the areoles or squamules round to angular, sometimes imbricate, 0.3–2.1 mm wide, ca 400–800 µm thick. Upper surface



FIG. 1. *Sarcogyne saphyniana* (holotype): Thallus with apothecia. Scale bar = 1 mm.

usually pale brown, sometimes dark brown, smooth, epruinose, sometimes slightly shiny. Lower surface corticated and dark, with a stipe. Upper cortex 35–55 μm thick, its upper layer reddish brown, lower layer hyaline. Algal layer, ≤ 280 μm thick, interrupted by wide hyphal bands, algal cells 7.5–18 μm in diam. Medulla white, prosoplectenchymatous, ≤ 385 μm thick, continuous with stipe. Apothecia 1–4 per thallus per unit, round to irregular, 0.3–1.4 mm wide, emerging from the thallus as they mature and projecting above the thallus. Disc reddish-brown, smooth to fissured, margin formed of the exciple, color blackish-brown or same color as disc. Exciple ≤ 45 μm wide, expanding around the disc ≤ 100 μm wide. Hymenium hyaline, 66–80 μm high, I+ blue. Epihymenium reddish-brown, ≤ 18 μm high. Paraphyses 2.0–2.5 μm wide, apices reddish-brown, barely expanded. Asci clavate, $40\text{--}7 \times 2\text{--}17$ μm . Ascospores hyaline, simple, broadly ellipsoid, $3.5\text{--}5.5 \times 2\text{--}2.5$ μm ($N = 50$). Subhymenium ≤ 50 μm high. Hypothecium, hyaline, ≤ 45 μm high. Pycnidia not observed. Lacking secondary metabolites.

ADDITIONAL SPECIMENS EXAMINED — CHINA. XINJIANG. Altay Mountains, Two-River Source Nature Reserve, $47^{\circ}35'N$ $88^{\circ}39'E$, alt. 1075 m, shale, 18 May 2014, A. Abbas, G. Sahadat 20140412 (XJU-NALH); Altay Mountain Grape Scenic Spot, $47^{\circ}48'N$ $88^{\circ}04'E$, alt. 917 m, shale, 16 May 2014, A. Abbas, G. Sahadat 20140418 (XJU-NALH); Tianshan Mountain, Xiao Q Zi Village, $44^{\circ}08'N$ $81^{\circ}42'E$, alt. 1490 m, sandstone, 30 Jul 2007, A. Abbas 20077730 (XJU-NALH); Shi Ren Gou, $43^{\circ}45'N$ $87^{\circ}50'E$, alt. 1345 m, 24 Jul 2013, sandstone, A. Abbas, G. Nazarbek, L. Nurtai 20137224 (XJU-NALH).

Sarcogyne saphyniana belongs to the *badiofusca* group (Knudsen et al. 2014) characterized by emergent lecideine apothecia with a non-carbonized margin. *Acarospora badiofusca* has a similarly low hymenium but differs in its uninterrupted algal layer. *Sarcogyne saphyniana* has an algal layer interrupted

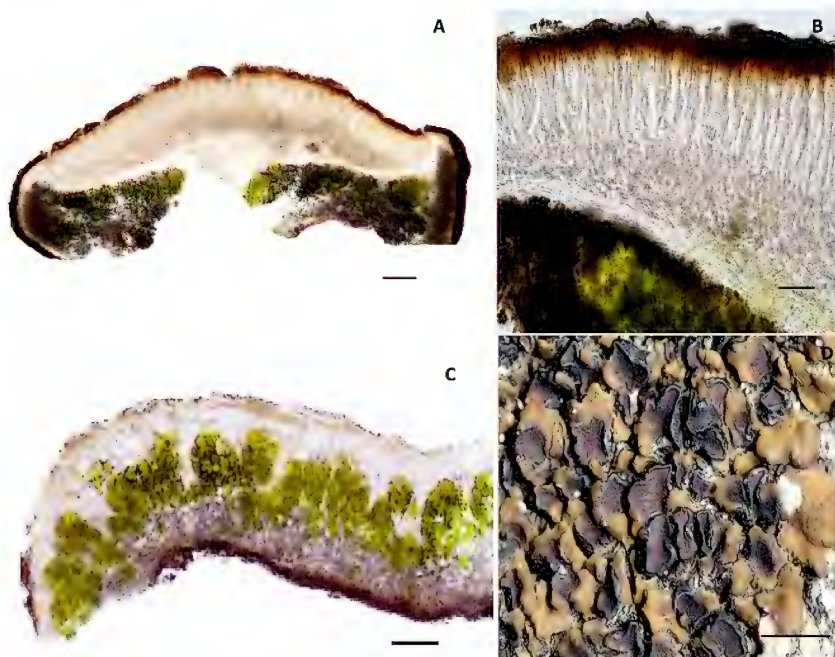


FIG. 2. *Sarcogyne saphyniana* (holotype): A, B. Cross-section of apothecia; C. Interrupted algal layer in thin section of the thallus; D. Thallus with apothecia. Scale bars: A= 50 μ m; B = 20 μ m; C = 100 μ m; D = 1 mm.

by wide hyphal bands as does another member of the *badiofusca* group, the European species *A. irregularis* H. Magn., which differs in its higher (80–140 μ m) hymenium (Knudsen et al. 2014). The third member of the *badiofusca* group, *A. boulderensis* H. Magn. from North America, is distinguished by its higher hymenium and an algal layer interrupted by narrow hyphal bands that do not radically break up the algal layer into palisades and clumps of algal cells (Knudsen et al. 2014). *Acarospora cervina* is distinguished from *S. saphyniana* by its higher (85–100 μ m) hymenium, narrowly ellipsoid ascospores (4–5 $\times$ 2 μ m), and pruina on the margins or entire thallus (Magnusson 1929; for an example of the typical *A. cervina* phenotype, see Wirth et al. 2013). All species discussed above have an I+ blue hymenium. Of the five *Sarcogyne* species previously reported in China, three have been collected in Xinjiang (Wei 1991, Abbas 1998, 2000).

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***Mirandina uncinata* sp. nov. from submerged leaves from Brazil**

PATRÍCIA OLIVEIRA FIUZA¹, JOSIANE SANTANA MONTEIRO¹,
LUÍS FERNANDO PASCHOLATI GUSMÃO^{1*} & RAFAEL F. CASTAÑEDA-RUIZ²

¹Universidade Estadual de Feira de Santana, Depto. de Ciências Biológicas, Lab. de Micologia,
Avenida Transnordestina s/n, Bairro Novo Horizonte, 44036-900, Feira de Santana, Brazil

²Inst. de Investigaciones Fundamentales en Agricultura Tropical 'Alejandro de Humboldt',
Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P. 17200

* CORRESPONDENCE TO: lgusmao@uefs.br

ABSTRACT — A new species, *Mirandina uncinata*, collected on submerged leaves of *Calophyllum brasiliense* in the Brazilian semiarid region, is described and illustrated. *Mirandina uncinata* is characterized by polyblastic, integrated, terminal, sympodially extended conidiogenous cells and cylindrical-fusiform, uncinata toward the apex, 5–7-septate, pale brown conidia.

KEY WORDS — freshwater fungi, conidial fungi, taxonomy

Introduction

An undescribed *Mirandina* species was collected during an investigation of conidial fungi associated with submerged decaying leaves of *Calophyllum brasiliense* in a riparian forest of Chapada Diamantina in Brazil's semiarid region. It is described here as new.

Materials & methods

Samples of submerged leaves of *C. brasiliense* were collected in Piatã, Bahia State, from November 2013 to January 2015. The material was placed in plastic bags and taken to the laboratory and processed according to Castañeda-Ruiz (2005). The samples were washed, placed in Petri dish moist chambers, and stored in a polystyrene box with sterile water plus glycerol for 30 days. Slide mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were made at a magnification of $\times 1000$. Microphotographs were obtained with an Olympus microscope (model BX51) equipped with bright field and Nomarski interference optics. The specimens and

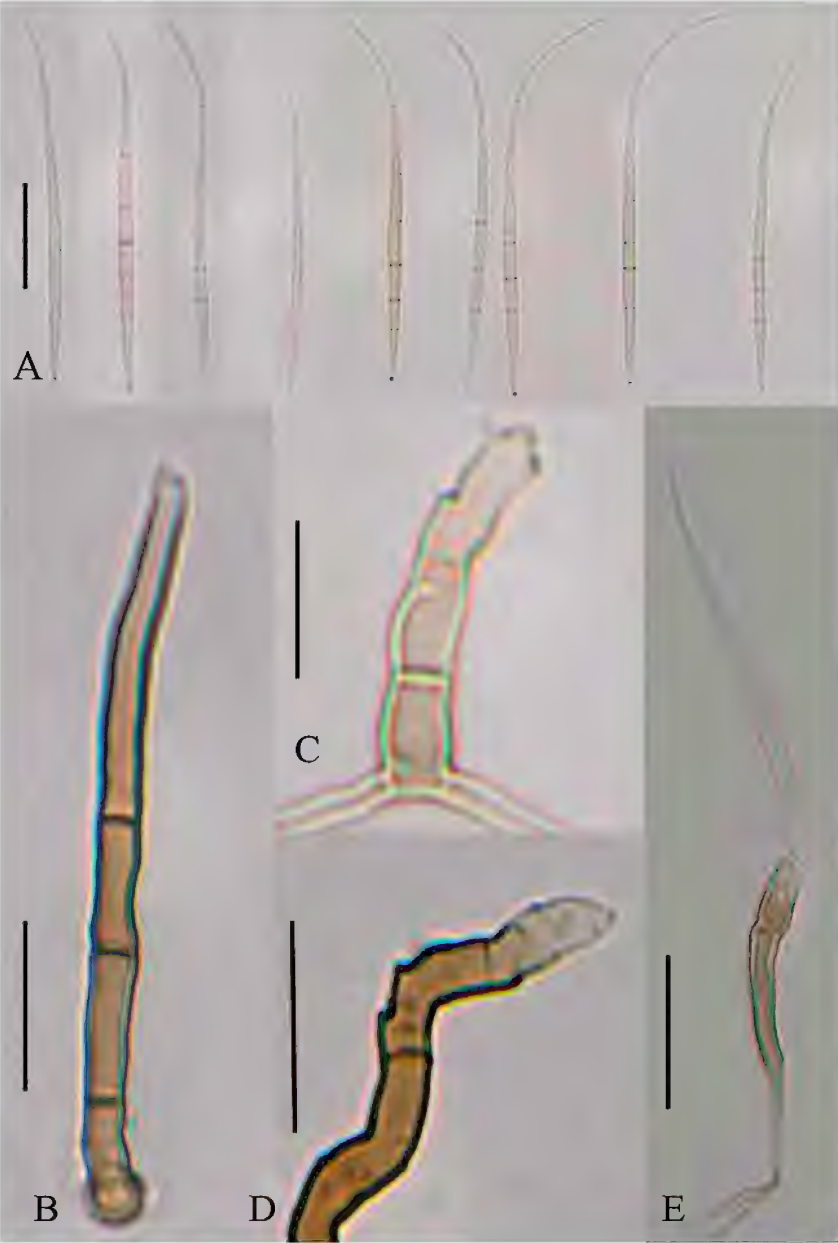


FIG. 1. *Mirandina uncinata* (ex HUEFS 216003). A. Conidia; B–D. Conidiophores and conidiogenous cells; E. Conidiophore and conidium. Bars: A, B, D, E = 20 μ m; C = 10 μ m.

holotype are deposited in the Herbarium of Universidade Estadual de Feira de Santana, Brazil (HUEFS).

Taxonomy

Mirandina uncinata Fiuza, J.S. Monteiro, R.F. Castañeda & Gusmão, **sp. nov.** FIG. 1

MYCOBANK MB813056

Differs from *Mirandina flagelliformis* by cylindrical-fusiform, pale brown conidia that are mostly 5–7-septate and uncinata toward the apex.

TYPE: Brazil, Bahia, Piatã, Serra da Tromba, 13°07'S 41°50'W, on submerged decaying leaves of *Calophyllum brasiliense* Cambess. (*Clusiaceae*), 21. XI. 2014, coll. P.O. Fiuza (Holotype: HUEFS 216003).

ETYMOLOGY: Latin, *uncinata*, referring to the hooked conidia.

COLONIES on the natural substratum effuse, hairy, hypophyllous, brown. Mycelium mostly immersed in the substrate. CONIDIOPHORES distinct, single, unbranched, cylindrical, straight to geniculate toward the apex, 2–7-septate, brown at the base becoming paler to the apex, smooth, $20\text{--}100 \times 3\text{--}5\ \mu\text{m}$. Conidial secession schizolytic. CONIDIOGENOUS CELLS polyblastic, integrated, mostly terminal, sometimes intercalary, indeterminate, sympodially elongated, slightly denticulate, brown to pale brown, $20\text{--}25 \times 3\text{--}4\ \mu\text{m}$, slightly cicatrized at the loci. CONIDIA solitary, cylindrical-fusiform, attenuate, truncate at the base, rostrate, uncinata toward the apex, (1–)5–7(–11)-septate, pale brown, smooth, $40\text{--}95 \times 2\text{--}4.5\ \mu\text{m}$.

ADDITIONAL SPECIMENS EXAMINED: BRAZIL, PARÁ, Belém, Ilha do Combu, 1°29 S 48°25 W, on submerged decaying twig 29 X. 2011, coll. J.S. Monteiro (HUEFS 216005); Parque Estadual de Utinga, 1°25 S 48°27 W, on submerged decaying twig, 30 VII. 2012, coll. J.S. Monteiro (HUEFS 216004).

NOTE: The genus *Mirandina* G. Arnaud ex Matsush., typified by *M. corticola*, is characterized by distinct, unbranched, brown conidiophores and polyblastic, denticulate, integrated, sympodial elongated conidiogenous cells. The conidia are cylindrical, fusiform to obclavate, unicellular or septate, hyaline, and subhyaline (Kirk 1986). Ten species have been accepted in *Mirandina* (Castañeda-Ruiz & Kendrick 1991, Castañeda-Ruiz et al. 1997, de Hoog 1985, Kirk 1986, Matsushima 1975, 1980, 1987; Matsushima & Matsushima 1996, Paulus et al. 2003, Ma et al. 2015). *Mirandina uncinata* resembles *M. flagelliformis* Matsush., which differs by its narrowly obclavate hyaline conidia that are flagelliform toward the apex and slightly constricted at the septa (Matsushima 1987, Matsushima & Matsushima 1996).

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***Pleurothecium bicoloratum* & *Sporidesmiopsis pluriseptata* spp. nov. from Brazil**

JOSIANE SANTANA MONTEIRO¹, LUIS FERNANDO PASCHOLATI GUSMÃO^{1*}
& RAFAEL F. CASTAÑEDA-RUIZ²

¹ Universidade Estadual de Feira de Santana,

Avenida Transnordestina, s/n, Novo Horizonte, 44036-900, Feira de Santana, Brazil

² Instituto de Investigaciones Fundamentales en Agricultura 'Alejandro de Humboldt' (INIFAT),

Calle 1 Esq. 2, C.P. 17200, Santiago de Las Vegas, C. Habana, Cuba

* CORRESPONDENCE TO: lgusmao@uefs.br

ABSTRACT — Two new species of microfungi, *Pleurothecium bicoloratum* and *Sporidesmiopsis pluriseptata*, found on decaying plant materials are described and illustrated. *Pleurothecium bicoloratum*, also collected in Venezuela, is distinguished by broad allantoid, bicolored, 3-septate, smooth conidia with dark olivaceous-brown central cells and hyaline ends; *S. pluriseptata*, thus far known only from Brazil, is characterized by obclavate, smooth, dirty brown conidia with up to 23 septa.

KEY WORDS — asexual fungi, taxonomy, leaf litter

Introduction

During a survey of hyphomycetes associated with plant litter from the Amazon forest (Amapá state) and semi-arid region (Bahia state), two undescribed *Pleurothecium* and *Sporidesmiopsis* species were collected. The specimens, which could not be assigned to any species currently accepted in those genera, are described here as new.

Materials & methods

Leaf and wood litter samples were transported to the laboratory where they were placed in Petri dish moist chambers and stored in a 170 L polystyrene box with 200 mL sterile water plus 2 mL glycerol, at 25°C for 30 days (Castañeda-Ruiz 2005). Mounts were prepared in polyvinyl alcohol, lactic acid, and phenol (PVL) and examined and

photographed using an Olympus BX51 microscope equipped with bright field and Nomarski interference optics. All measurements were made at a magnification of $\times 1000$. The type specimens are deposited in the Herbarium of Universidade Estadual de Feira de Santana, Brazil (HUEFS).

Taxonomy

Pleurothecium bicoloratum R.F. Castañeda, J.S. Monteiro & Gusmão sp. nov.

FIGS 1–3A

MYCOBANK MB 813040

Differs from all other *Pleurothecium* species by its bicolored conidia with dark olivaceous brown to black central cells and hyaline end cells.

TYPE: Brazil, Bahia State, Abaíra, Distrito de Catolés, Mata do Cigano, 13°15'S 41°54'W, on decaying wood of an unidentified plant, 09.I.2015; coll. J.S. Monteiro (Holotype: HUEFS 215982).

ETYMOLOGY: Latin, *bicoloratum*, referring to the bicolored conidia.



FIG. 1. *Pleurothecium bicoloratum* (ex holotype HUEFS 215982). Conidiophores, conidiogenous cells, and conidia. Bar = 20 μm .

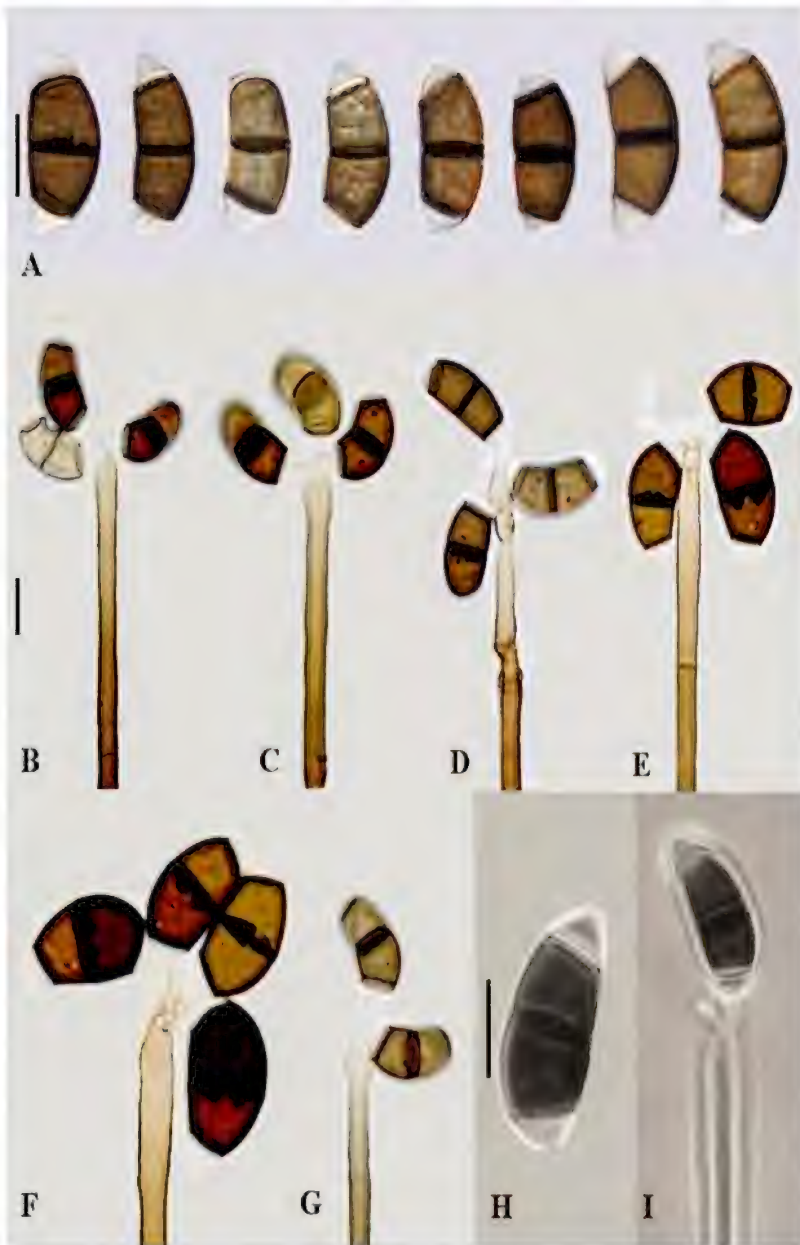


FIG. 2. *Pleurothecium bicoloratum* (ex holotype HUEFS 215982). A, H. Conidia; B–G, I. Conidiogenous cells and conidia. Bars = 10 µm.

COLONIES effuse, hairy dark brown or black. Mycelium mostly immersed in substrate. CONIDIOPHORES macronematous, mononematous, erect, straight to slightly flexuous toward the apex, single, 5–10-septate, smooth, dark brown below, pale brown toward the apex, $300\text{--}470 \times 5\text{--}7.5 \mu\text{m}$. CONIDIOGENOUS CELLS polyblastic, integrated, terminal, sometimes becoming intercalary, $20\text{--}90 \times 4\text{--}5 \mu\text{m}$ sympodially elongated, denticulate, denticles narrow cylindrical, hyaline, $3\text{--}4 \times 1\text{--}1.5 \mu\text{m}$. Conidial secession schizolytic. CONIDIA solitary, broad allantoid, smooth, 3-septate, bicolor, with dark olivaceous-brown to black central cells, hyaline end cells, smooth, $19\text{--}24 \times 7\text{--}12 \mu\text{m}$.

ADDITIONAL SPECIMEN EXAMINED: VENEZUELA, ARAGUA STATE: Forest near Colonia “Tovar”, $10^{\circ}25'N$ $67^{\circ}17'W$, on decaying petiole of *Bactris setulosa* H. Karst. (*Areaceae*), 16.XII.2003; coll. J. Mostacero (HUEFS 216011).

NOTE: Höhnelt (1919) established *Pleurothecium* for *P. recurvatum* (Morgan) Höhn. The genus is characterized by distinct brown conidiophores and polyblastic sympodially extended denticulate conidiogenous cells. The conidia are solitary, unicellular or septate, hyaline or pigmented (Arzanlou et al. 2007, Cooper 2005, Goos 1969, Matsushima 1975, 1980, Matsushima & Matsushima 1996, Réblová et al. 2012, Subramanian & Bhat 1989, Wu & Zhang 2009). *Pleurothecium leptospermi* J.A. Cooper and *P. recurvatum* are superficially similar to *P. bicoloratum* in having paler end cells and darker central cells, but *P. leptospermi* has brown denticles and fusiform 3-septate brown smaller ($15\text{--}18 \times 4\text{--}5 \mu\text{m}$) conidia, while the allantoid conidia of *P. recurvatum* are hyaline initially and narrower ($18\text{--}23\text{--}(30) \times 5\text{--}7 \mu\text{m}$). The conidia of other described *Pleurothecium* species clearly differ from *P. bicoloratum* (see FIG. 3). *Pleurothecium magnum* Subram. & Bhat (Subramanian & Bhat 1989) should be excluded from *Pleurothecium*: it more closely resembles *Kylindria* species and is distinguished by sympodial extensions of the inner conidiogenous cell layers, some enteroblastic percurrent regenerations, and conidia eccentrically attached to flattened denticulate conidiogenous loci—none of which are characters assigned to *Pleurothecium*.

Key to *Pleurothecium* species

- 1. Conidia unicellular 2
- 1. Conidia septate 3
- 2. Conidia ellipsoidal to oblong or obovoid, hyaline, $5\text{--}8 \times 2.5\text{--}3 \mu\text{m}$... *P. semifecundum*
- 2. Conidia narrowly obovoid, pale brown, $8.5\text{--}13.5 \times 4\text{--}5 \mu\text{m}$ *P. obovoideum*
- 3. Conidia 1-septate 4
- 3. Conidia with more than one septum 5
- 4. Conidia clavate, pale brown, $15\text{--}32 \times 3\text{--}4 \mu\text{m}$ *P. clavatum*
- 4. Conidia cylindrical, hyaline to pale brown, $14\text{--}21 \times 1.5\text{--}2 \mu\text{m}$ *P. malayense*

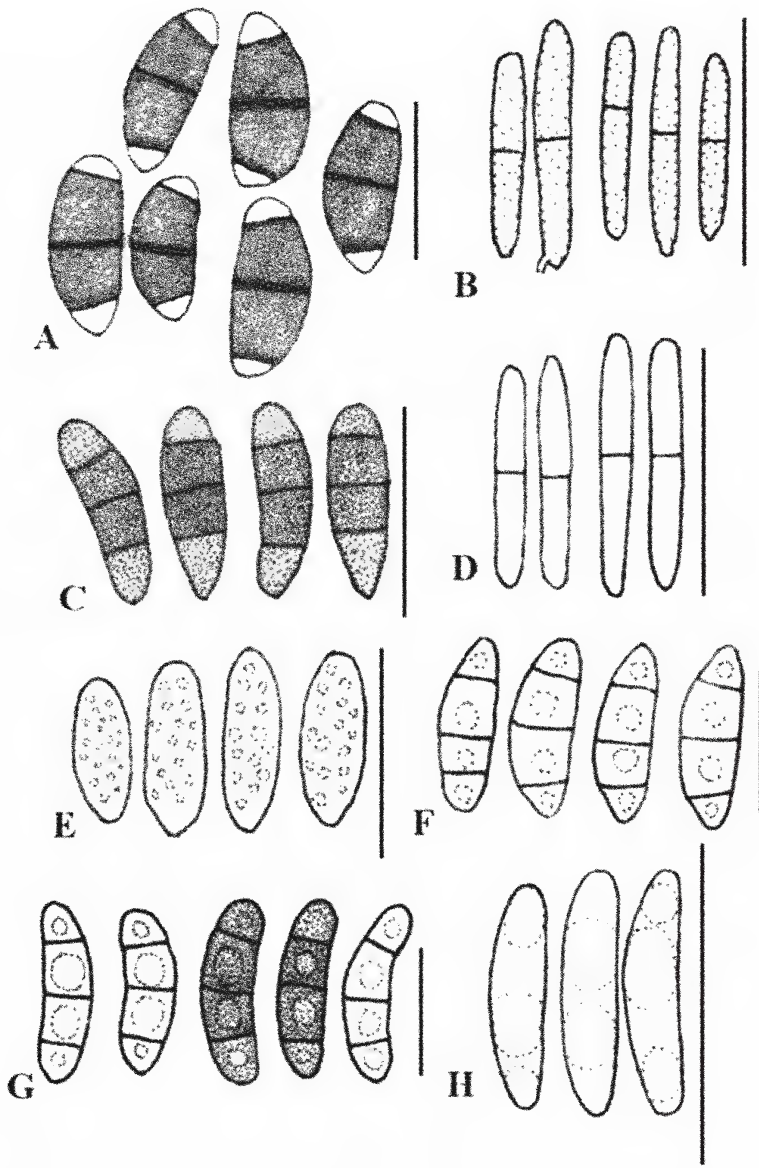


FIG. 3. Representative conidia of *Pleurothecium* spp. (re-drawn from the literature). A. *P. bicoloratum*. B. *P. clavatum*. C. *P. leptospermi*. D. *P. malayense*. E. *P. obovoideum*. F. *P. pulneyense*. G. *P. recurvatum*. H. *P. semifecundum*. Bars = 20 μm.

5. Conidia 3-septate, always hyaline, cylindrical to fusiform, slightly curved, guttulate,
23–30 × 7–8 µm *P. pulneyense*
5. Conidia 3-septate, sometimes or always pigmented 6
6. Conidia fusiform, curved, brown with median cells darker than end cells,
15–18 × 4–5 µm *P. leptospermi*
6. Conidia allantoid 7
7. Conidia always with dark olivaceous-brown to black median cells and
hyaline end cells, 16–27 × 7–12 µm *P. bicoloratum*
7. Conidia at first hyaline, later pale brown with median cells darker than
the end cells, 18–23(–30) × 5–7 µm *P. recurvatum*

***Sporidesmiopsis pluriseptata* J.S. Monteiro, Gusmão & R.F. Castañeda sp. nov.**

FIG 4

MYCOBANK MB 813041

Differs from all other *Sporidesmiopsis* spp. by its larger and multiseptate conidia.

TYPE: Brazil. Amapá State: Serra do Navio, 00°54'N 51°59'W, on decaying leaves of *Elaeis* sp. (*Arecaceae*), 14.X.2014; coll. H.M.P. Sotão (**Holotype**: HUEFS 216009).

ETYMOLOGY: Latin, *pluri*-, meaning many several, + *-septata*, refers to the septa.

COLONIES effuse, hairy, black. Mycelium mostly immersed in substrate. CONIDIOPHORES macronematous, mononematous, erect, straight, branched above, cylindrical, ≤22-septate, sometimes with 1–2 enteroblastic regenerations, smooth, black, 160–320 × 6–10 µm, 0–7-septate, branches more or less cylindrical, sometimes slightly sinuate, dark brown, 7–30 × 4–5 µm. CONIDIOGENOUS CELLS monoblastic, mostly discrete, lateral, determinate, sometimes integrated, terminal, doliiform or shortly cylindrical, dark brown, smooth, 3–7 × 4–5 µm. Conidial secession schizolytic. CONIDIA solitary, obclavate, straight or curved, acropleurogenous, 11–23-euseptate, brown, pale brown toward the apex, smooth, 80–130 × 6–8.5 µm, smooth, sometimes with a rudimentary, hyaline, mucous tunica at the apical cell.

NOTE: Subramanian & Bhat (1989) established *Sporidesmiopsis* for *S. malabarica* Subram. & Bhat. The genus is characterized by conidiophores that are distinct, erect, branched toward the apex, and conidiogenous cells that are monoblastic, discrete, and short cylindrical or doliiform (Xia et al. 2014). *Sporidesmiopsis dennisii* (J.L. Crane & Dumont) Bhat et al., which is superficially similar to *S. pluriseptata*, is distinguished by its 10–15-septate, smooth or verruculose, dark brown conidia, paler toward the apex, 69–92 × 11–13 µm (Bhat & Kendrick 1993, Crane & Dumont 1978).

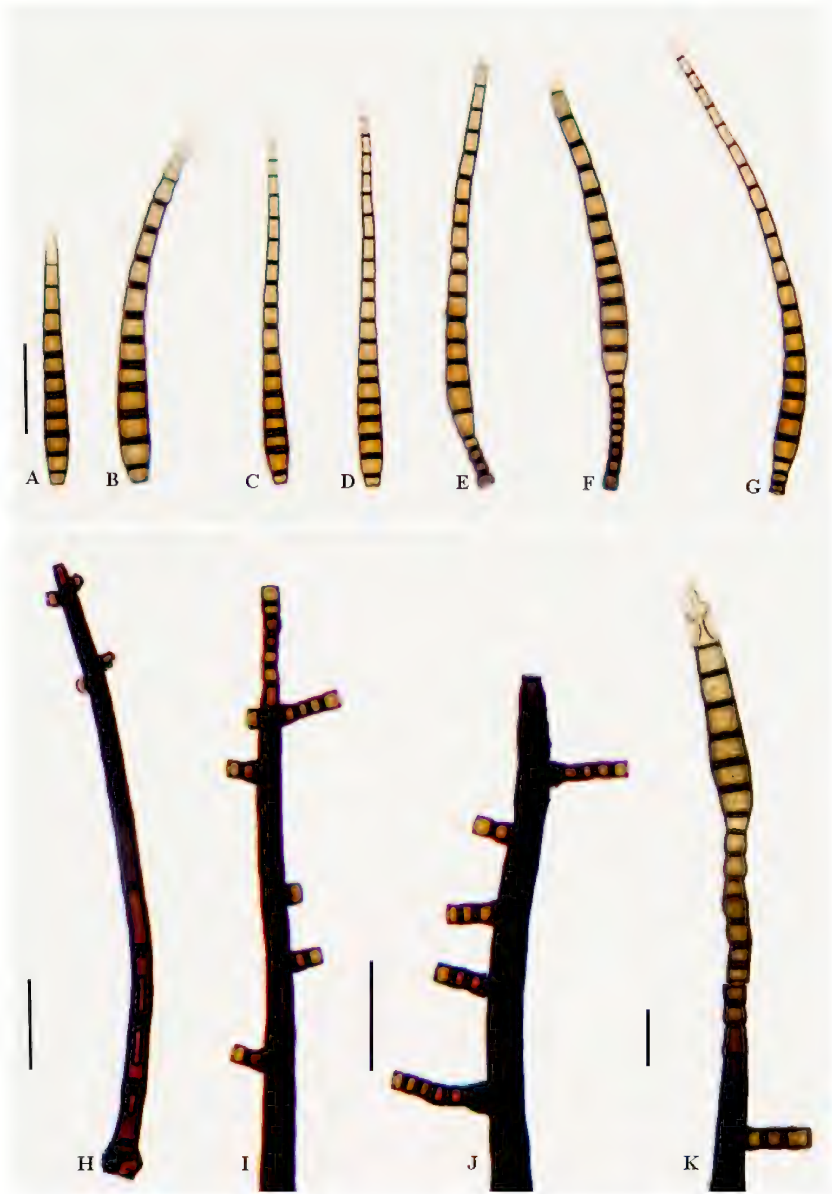


FIG. 4. *Sporidesmiopsis pluriseptata* (ex holotype HUEFS 216009). A–G. Conidia; H–K Conidiophores, conidiogenous cells and conidium. Bars: A–J = 20 μ m; K = 10 μ m.

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The authors express their sincere gratitude to Dr. De-Wei Li and Dr. Xiu-Guo Zhang for their critical review of the manuscript. The authors thank the National Council for Scientific and Technological Development (CNPq) (Proc. 142014/2011–7 and 303062/2014–2) and “Programa de Pesquisa em Biodiversidade do Semiárido (PPBio Semiárido -CNPq/MCTI) for supporting this study. JSM thank the Postdoctoral fellowship (CAPES - 071/2012). RFCR is grateful to Cuban Ministry of Agriculture and “Programa de Salud Animal y Vegetal”, project P131LH003033 Cuban for facilities. We acknowledge the assistance provided by Dr. P.M. Kirk and Drs. V. Robert and G. Stegehuis through the Index Fungorum and MycoBank websites. Dr. Lorelei Norvell’s editorial and Dr. Shaun Pennycook’s nomenclatural reviews are greatly appreciated.

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New leptonioid species from New South Wales and northeastern Queensland, Australia

DAVID L. LARGENT^{1*}, KERRI L. KLUTING²,
NOELLE M. ANDERSON³ & SARAH E. BERGEMANN³

¹Biological Sciences, Humboldt State University, 1 Harpst St, Arcata CA 95521, USA

²Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University,
Uppsala, SE-752 36, Sweden

³Evolution & Ecology Group, Biology Department, Middle Tennessee State University,
PO Box 60, Murfreesboro TN 37132, USA

* CORRESPONDENCE TO: mrp@humboldt1.com

ABSTRACT — *Leptonia subpanniculus*, *L. newlingii*, *L. sabulosa*, and *L. substricta* are described as new species based on morphological and molecular characters. Phylogenetic relationships and morphological similarities are discussed for these and other closely related species.

KEY WORDS — Basidiomycota, Entolomaceae, taxonomy

Introduction

The morphologically defined genus *Leptonia* (Fr.) P. Kumm. is polyphyletic (Co-David et al. 2009). In the most recent taxonomic study of *Leptonia*, phylogenetic analyses strongly support *Entoloma euchroum* (Pers.) Donk [≡ *Leptonia euchroa* (Pers.) P. Kumm.], the type species of *Leptonia* and *Entoloma* subg. *Leptonia* (Fr.) Noordel. emend. Morozova et al. (2014), in a clade separated from the *Inocephalus*-*Cyanula* and *Nolanea*-*Claudopus* clades (Morozova et al. 2014). These authors have emended *Entoloma* subg. *Leptonia* to make it monophyletic and consistent with *Entoloma* sect. *Leptonia* (Noordeloos 2004) and *Leptonia* subg. *Leptonia* (Largent 1994). *Leptonia* subg. *Leptonia* sensu Largent is a diverse subgenus that includes species that are mycenoid, collybioid, or tricholomatoid; have a conico-convex or plano-convex pileus, a fibrillose-striate or flocculose-scaly (rarely almost smooth) stipe, lamellae with

sterile, fertile or heterogeneous edges, cheilocystidia present or absent, weakly to sharply angled (almost nodulose) basidiospores, a more or less a trichoderm pileipellis of inflated elements with intracellular (and often with additional encrusting) pigments, and clamp connections; and lack brilliant granules.

Here we describe and illustrate four new species from Australia: *L. sabulosa*, *L. subpanniculus*, *L. substricta*, and *L. newlingii*. Our molecular phylogeny supports these species and *L. ambigua* from Australia (Largent et al. 2013b) within *Leptonia* subg. *Leptonia* sensu Largent (1994). We also discuss the morphological and phylogenetic relationships among species in this clade.

Materials & methods

Macromorphological and micromorphological features

Techniques and equipment for collecting and describing basidiomata in the field and GPS coordinates follow Largent et al. (2011a,b). Kornerup & Wanscher (1978) color terms and factors determined from mathematical analyses used in the descriptions are in Largent et al. (2013a,b). Digitized microphotographs were made using a Lumenera Infinity 2-5C ccd digital camera attached to the trinocular head of a compound microscope; all microscopic measurements were obtained using Infinity Analyze software 6.5.2. All collections from New South Wales cited in 'Additional collections examined' were deposited in The Plant Pathology Herbarium, Orange Agricultural Institute, Orange, New South Wales, Australia (DAR) while those from Queensland were deposited in the Australian Tropical Herbarium, Cairns, Queensland, Australia (CNS). All holotype and isotype collections are deposited in the herbaria designated using acronyms from Thiers (2012).

DNA sequences and phylogenetic analyses

DNAs were extracted from silica-dried tissues according to Largent et al. (2011b). The nuclear large subunit ribosomal RNA (nrLSU) and the second largest subunit of the ribosomal RNA polymerase (RPB2) genes were PCR amplified following protocols described in prior publications (Largent et al. 2011a,b, 2013a,b). Partial mitochondrial small subunit ribosomal RNA (mtSSU) sequences were obtained using two different primer combinations: either 1) with MS1 and MS2 following White et al. (1990) or 2) by modifying the MS1 and MS2 primers to increase the success of PCR amplification (MS1-RhoF2, 5' to 3' – GTC AAT GCT CGM AAG AGT GAA CTA and MS2-RhoR1, 5' to 3' – ACA TGC TTC ACT YCG TTT GCT). The PCR protocol using the newly-developed primers is as follows: initial incubation at 94°C for 1 min, 39 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min 30 sec, and a final temperature of 72°C for 7 min. Sequences were generated on an ABI3130xl following the sequencing and precipitation protocols in Largent et al. (2011b). After sequencing, forward and reverse sequences were edited and all ambiguities in chromatograms were visually confirmed using Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, MI).

TABLE 1. Collections used in the phylogenetic analyses.

New sequences generated for this study are shown in bold. Square bracketed annotations indicate species names applied in GenBank that differ from those in the phylogram (FIG. 1).

SPECIES	COLLECTION IDENTIFIER	GENBANK ACCESSION No.		
		mtSSU	LSU	RPB2
<i>Alboleptonia angustospora</i> [= <i>A. aff. sericella</i>]	MCA1978	GU384583	GU384609	GU384632
<i>A. sericella</i>	DLL9524	KR869912	KR869940	KR869961
<i>A. stylophora</i>	DLL9805	KR869911	KR869939	KR869960
<i>Catathelasma imperiale</i>	11CA001A	KR869913	KR869941	KC816994
<i>Claudopus minutoincanus</i>	DLL9871	HQ731511	HQ731514	HQ731517
<i>C. parasiticus</i> [= <i>Entoloma parasiticum</i>]	20	GQ289317	GQ289177	GQ289248
	10617 TJB	KR233902	KR233872	KR233924
<i>Claudopus</i> sp.	DLL9735	KR869910	KR869938	KR869959
<i>C. viscosus</i>	DLL9788	HQ731513	HQ731516	HQ731518
<i>Clitocella fallax</i> [= <i>Rhodocybe fallax</i>]	25668OKM	KR869909	AF261283	KC816937
<i>Clitopilopsis hirneola</i> [= <i>Rhodocybe hirneola</i>]	REH8490	GU384587	GU384611	KC816904
<i>Clitopilus</i> aff. <i>hobsonii</i>	DLL9586	KJ021688	KJ021698	KC816912
<i>Cl. apalus</i>	26394WAT	KR869907	KR869936	KC816906
<i>Entocybe haastii</i> [= <i>Entocybe</i> sp.]	DLL10087	JQ793645	JQ793652	JQ793659
<i>Entoloma abortivum</i>	6693 TJB	GU384595	GU384616	GU384642
	5772 TJB	KR233897	KR233869	KR233926
<i>E. allochroum</i>	JVG 1060902-1	KC898488	KC898522	—
<i>E. caespitosum</i>	GDGM 27564	JQ993070	JQ320130	JQ993078
<i>E. callichroum</i> var. <i>venustum</i>	L Wo E17/10	KC898490	KC898523	—
<i>E. chytrophilum</i>	L Dahncke 855	KC898479	KC898519	—
	LE262994	KC898480	KC898520	—
<i>E. dichroum</i>	JVG 1070821-4	KC898486	KC898527	—
	LE234260	KC898487	KC898528	—
<i>E. discoloratum</i>	DLL10217	JQ793646	JQ793653	JQ793660
<i>E. euchroum</i>	LE262995	KC898483	KC898516	—
<i>E. eugenei</i>	LE253771	—	KC898529	—
	LE254347	—	KC898530	—
<i>E. ferruginans</i>	11CA032	KJ021689	KJ021699	KJ021693
<i>E. fragilum</i>	MCA2415	KJ021690	KJ021700	KJ021694
<i>E. indoviolaceum</i>	PM700	GQ289312	GQ289172	GQ289243
<i>E. lampropus</i>	LE262991	KC898470	KC898505	—
	UPS:F-176490	KC898471	KC898506	—
<i>E. lepidissimum</i>	LE254871	KC898493	KC898531	—
<i>E. myrmecophilum</i>	231	GQ289314	GQ289174	GQ289245
<i>E. nidorosum</i>	9971 TJB	GU384596	GU384617	GU384643
<i>E. pallideradicatum</i>	255	GQ289316	GQ289176	GQ289247
<i>E. pallidocarpum</i>	GDGM 28828	JQ993074	JQ410331	JQ993080
<i>E. percoelestinum</i>	LE254327	KC898496	KC898526	—

Continued on p. 156

TABLE 1, continued

SPECIES	COLLECTION IDENTIFIER	GENBANK ACCESSION No.		
		mtSSU	LSU	RPB2
<i>E. placidum</i>	JVG1060830-6	KC898482	KC898515	—
<i>E. prunuloides</i>	40	GQ289324	GQ289184	GQ289255
<i>E. pygmaeopapillatum</i>	32	GQ289325	GQ289185	GQ289256
<i>E. readiae</i>	102	GQ289326	GQ289186	GQ289257
<i>E. rhodopolium</i>	8	GQ289327	GQ289187	GQ289258
<i>E. sublaevisporum</i>	LIP JVG 1070823T	KC898478	KC898518	—
<i>E. tectonica</i>	i15	GQ289336	GQ289196	GQ289266
<i>E. tjallingiorum</i> var. <i>alnetorum</i> [≡ <i>E. alnetorum</i>]	G00111402	KC898473	KC898508	—
<i>E. tjallingiorum</i> var. <i>laricinum</i>	LE254343	KC898477	KC898513	—
<i>E. tjallingiorum</i> var. <i>tjallingiorum</i>	UPS:F-016378	KC898474	KC898509	—
	LE254317	KC898475	KC898511	—
<i>E. transmutans</i>	155	GQ289340	GQ289200	GQ289268
<i>E. undatum</i>	18	GQ289342	GQ289202	GQ289270
<i>E. valdeumbonatum</i>	189	GQ289343	GQ289203	GQ289271
<i>E. vezzenaense</i>	241	GQ289344	GQ289204	GQ289272
<i>E. violaceotinctum</i>	DLL10088	JQ793643	JQ793650	JQ793657
<i>Inocephalus hypipamee</i>	DLL9981	JQ624603	JQ624608	KR869958
	DLL10071	JQ624604	JQ624609	JQ624616
“ <i>I. lactifluus</i> ”	7962 TJB	—	AF261304	—
<i>I. luteus</i>	6562 TJB	—	KR869944	—
<i>I. plicatus</i>	DLL9691	JQ624605	JQ624610	JQ624617
<i>I. virescens</i>	DLL9972	KR869908	KR869937	KR869957
<i>Lepista nuda</i>	11CA041	KJ021692	KJ021705	KJ136110
<i>Leptonia ambigua</i>	DLL9872	JQ756399	JQ756414	JQ756429
<i>L. boardinghousensis</i>	DLL10086	JQ756398	JQ756413	JQ756428
<i>L. coelestina</i> [≡ <i>Entoloma coelestinum</i>]	LE258103	KC898494	KC898524	—
<i>L. decolorans</i>	DLL9533	KR233883	KR233852	KR233914
<i>L. exilis</i>	DLL9530	KR869906	KR869935	KR869956
<i>L. newlingii</i>	DLL10004	KR869897	KR869926	—
	DLL10026	KR869898	KR869927	KR869951
<i>L. odorifera</i>	6534 TJB	KR233899	KR233870	KR233927
<i>L. omphalinoides</i>	DLL9800	JQ756401	JQ756417	JQ756432
<i>L. poliopus</i>	DLL10209a	JQ756402	JQ756418	JQ756433
<i>L. sabulosa</i>	DLL10058	KR869895	KR869924	KR869950
	DLL10427	KR869896	KR869925	—
<i>L. subpanniculus</i>	DLL9647	KR869891	KR869919	KR869946
	DLL9668	KR869892	KR869921	KR869947
	DLL9697	KR869893	KR869922	KR869948
	DLL10070	KR869889	KR869917	—
	DLL10227	—	KR869920	—
	DLL10260	KR869890	KR869918	—
<i>L. substricta</i>	DLL9852	KR869894	KR869923	KR869949

TABLE 1, cooncluded

SPECIES	COLLECTION IDENTIFIER	GENBANK ACCESSION No.		
		mtSSU	LSU	RPB2
<i>L. trichomata</i>	11CA006	KR233873	KR233840	KR233903
<i>L. umbraphila</i>	DLL9640	JQ756407	JQ756422	JQ756438
	DLL9766	JQ756408	JQ756423	JQ756439
<i>Mycena</i> aff. <i>pura</i>	11CA007	KR869914	KR869942	KC816995
<i>Nolanea bicoloripes</i>	DLL9532	KR869904	KR869933	KR869954
<i>N. cetrata</i>	DLL9531	KF738927	KF738942	KF771346
<i>N. cf. conferenda</i>	11CA014	KF738935	KF738946	KF771351
<i>N. conferenda</i> [≡ <i>Entoloma conferendum</i>]	6	GQ289300	GQ289160	GQ289231
<i>N. hebes</i> [≡ <i>Entoloma hebes</i>]	46	GQ289310	GQ289170	GQ289241
<i>N. hirtipes</i>	DLL9525	KR869905	KR869934	KR869955
<i>N. rigidipes</i>	DLL9528	KR869902	KR869932	KR869953
<i>N. sericea</i>	DLL9527	KR233882	KR233851	KR233913
	11CA055	KF738933	KF738944	KF771349
<i>Nolanea</i> sp.	DLL9976	KR869903	KR869931	KR869952
<i>N. strictior</i> var. <i>isabellina</i> [≡ <i>Entoloma. strictius</i> var. <i>isabellinum</i>]	7710 TJB	GU384594	GU384618	GU384641
<i>Panellus stipticus</i>	11CA052	KR869915	KR869943	KC816996
<i>Pouzarella farinosa</i>	DLL9934	HQ876538	HQ876516	HQ876495
<i>P. lasia</i>	DLL9662	HQ876551	HQ876529	HQ876507
<i>P. pamiae</i>	DLL9794	HQ876539	HQ876517	HQ876496
<i>P. pilocystidiata</i>	DLL9848	HQ876542	HQ876520	HQ876499
<i>Rhodocybe pruinosostipitata</i>	MCA1492	GU384608	GU384627	GU384653
<i>R. roseiavellanea</i>	8130 TJB	KR869901	KR869930	KC816982
<i>R. spongiosa</i>	MCA2129	GU384604	GU384628	GU384657
<i>Rhodophana nitellina</i>	7861 TJB	KR869900	KR869929	KC816959
<i>Tricholoma flavovirens</i>	11CA038	KJ021691	KJ021704	KC816997
<i>Trichopilus porphyrophaeus</i>	6957 TJB	—	AF261290	—

A total of 281 sequences (fifty-nine newly generated for this study and 222 obtained from GenBank) were used to infer phylogenetic relationships (TABLE 1). Sequences were aligned with MAFFT v. 7 (default settings) and manually adjusted using Mesquite 2.75 (Mesquite Software Inc., Austin, TX). Intronic regions in the RPB2 and hyper-variable regions and large introns in the mtSSU were excised before analyses. Aligned sequences were 1,078 bp (nrLSU), 585 bp (mtSSU), and 1501 bp (RPB2), and six mtSSU and 28 RPB2 sequences were coded as missing data (TABLE 1). A Maximum Likelihood (ML) analysis with 1000 replicates and specifying a GTRGAMMA model in RAXML-HPC2 XSEDE 8.0.24 (Stamatakis 2006) using the CIPRES Science Gateway 3.1 (Miller et al. 2010) was conducted on a concatenated alignment of the three gene regions. Each gene region was partitioned separately and the RPB2 region was partitioned across all codon positions for a total of five partitions in the dataset. Supports for branches were generated using 1000 bootstrap (BS) iterations, and BS support values ≥ 70 were considered significant.

Results & discussion

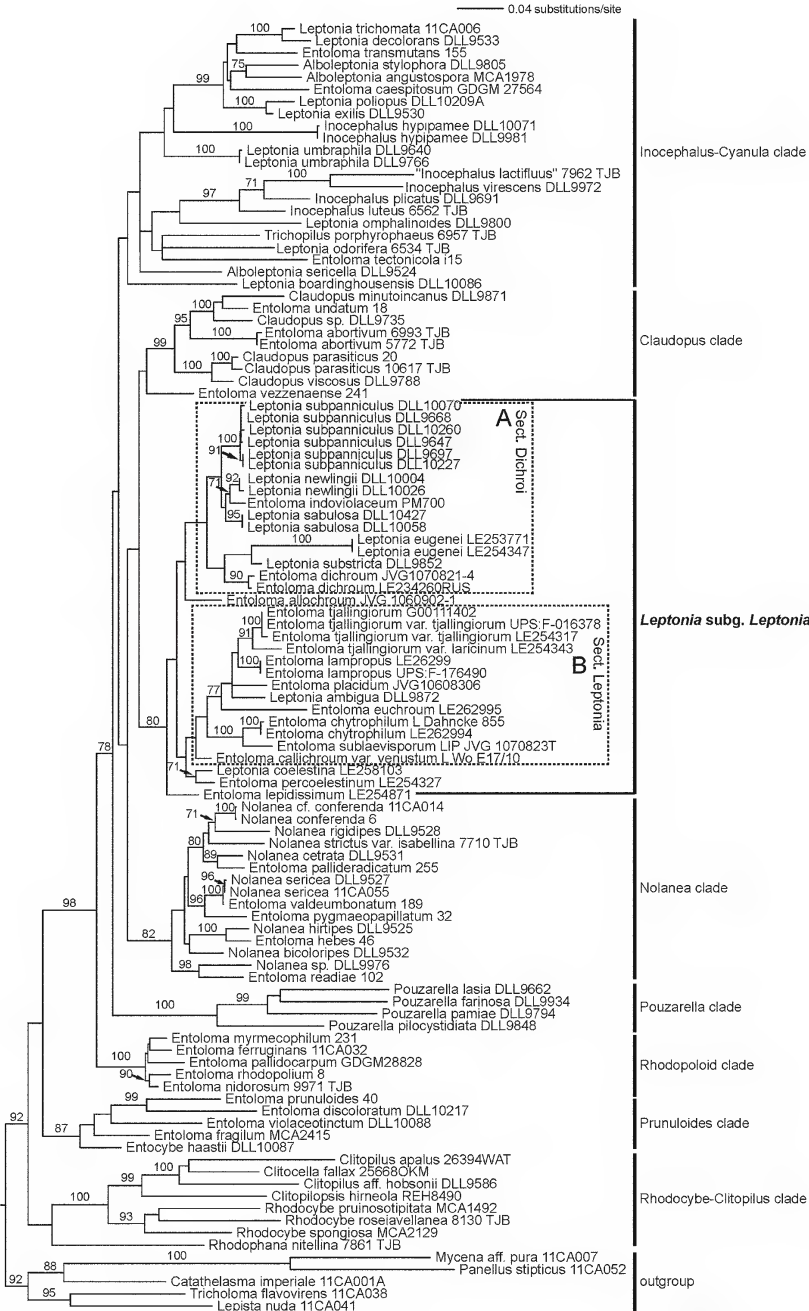
Our ML phylogenetic analysis (FIG. 1) statistically supports (BS = 80) monophyly of *Leptonia* subg. *Leptonia* ($\equiv$ *Entoloma* subg. *Leptonia*) and separates the *Leptonia* clade from other entolomatoid clades: *Inocephalus*-*Cyanula*, *Claudopus*, *Nolanea*, *Pouzarella*, *Prunuloides*, *Rhodopoloid*, and *Rhodocybe*-*Clitopilus* as previously demonstrated by Morozova et al. (2014). Within the *Leptonia* clade, Morozova et al. (2014) identified two subclades that correspond taxonomically to *Entoloma* sect. *Dichroi* O.V. Morozova et al. and *E.* sect. *Leptonia* as well as a number of species with unknown affinity to either section. Our analysis does not support the monophyly of these sections (FIG. 1: boxes A, B). Our results support the four new species described here, *L. ambigua* from Australia (Largent et al. 2013b), *E. indoviolaceum* Manim. & Noordel. from India, and the Eurasian species described by Morozova et al. (2014) all within *L.* subg. *Leptonia*.

Leptonia newlingii, *L. sabulosa*, and *L. subpanniculus* from Australia, *Entoloma indoviolaceum* from India, *E. dichroum* (Pers.) P. Kumm. from eastern and western Europe, *E. eugenei* Noordel. & O.V. Morozova from Russia, and *L. substricta* from Australia (FIG. 1: box A) all have violet or blue-black basidiomata and basidiospores with 5–7 more or less acute angles (PLATES. 2F, 4E, 6D, 8C). Although these seven species collectively resemble *E.* sect. *Dichroi* sensu Morozova et al. (2014), a phylogenetic relationship is not statistically supported (FIG. 1). Our analysis supports only one phylogenetic relationship for these species, with *L. newlingii* and *E. indoviolaceum* the most closely related (BS = 71, FIG. 1).

These seven species cluster into two groups—one with *Leptonia newlingii*, *L. sabulosa*, *L. subpanniculus*, and *Entoloma indoviolaceum* and the other with *E. dichroum*, *E. eugenei*, and *L. substricta*. However this clustering pattern was not statistically supported (FIG. 1). Within the two groups, the species have morphologically similar basidiospores. The group containing *L. sabulosa* has basidiospores that are distinctly angular but slightly rounded or with very slightly protruding angles, contrasting with the acutely angled basidiospores of *E. dichroum*, *E. eugenei*, and *L. substricta* that lack protruding angles.

Our analysis also places *L. ambigua* in a sub-clade with *E. euchroum* ($\equiv$ *L. euchroa*), *E. lampropus* (Fr.) Hesler, *E. placidum* (Fr.) Noordel., *E. tjallingiorum* var. *alnetorum* (Monthoux & Röllin) O.V. Morozova et al.,

FIGURE 1. Topology of the maximum-likelihood phylogram showing branches with statistical supports $\geq 70\%$ based on 1000 bootstrap replicates. Shaded species highlight the species from Australia described in this report. The labeled boxes show (A) *Entoloma* sect. *Dichroi* and (B) *E.* sect. *Leptonia* as defined by Morozova et al. (2014)—see also Results & discussion.



E. tjallingiorum var. *laricinum* O.V. Morozova et al., and *E. tjallingiorum* Noordel. var. *tjallingiorum* (BS = 77, FIG. 1). All these species have obscurely angled basidiospores and dark violet to blue-black squamulose pilei that become brown.

Taxonomy

Leptonia subpanniculus Largent & Bergemann, sp. nov.

PLATES 1, 2

MYCOBANK MB 813877

Differs from *Entoloma panniculus* by its dark purplish violet colors, mild and not distinctive taste and odor, and larger basidiospores.

TYPE — Australia, Queensland, Cook Region, Mt. Hypipamee National Park 17°25'35"S 145°29'16"E, in soil protected by roots, 9 March 2012, DL Largent 10260 (holotype BRI; isotype CNS; GenBank KR869890 (mtSSU), KR869918 (LSU)).

ETYMOLOGY — named for the similarity to *E. panniculus*.

BASIDIOMATA mycenoid to collybioid. PILEUS 10–38 mm broad, 5–13 mm high, at first conic, conic-campanulate, or campanulate (but not umbonate), expanding to broadly campanulate and umbonate to broadly convex with an obscure umbo, eventually convex without an umbo; when young covered entirely with minute erect dark purplish violet (17 or 18F6–7) squamules that with pileal expansion remain erect at the center and becoming semi-erect to appressed from margin to the edge; the squamules remaining dark purplish violet at the tips but becoming dark grayish violet (18E6) elsewhere and greyish violet (17C-D4–5) at the edge; the surface always dull and opaque, neither hyrophanous nor translucent-striate; the edge fibrillose, typically extending 1–1.5 mm beyond the lamellae, decurved then plane, even then eroded to crenulate. CONTEXT 1.0–1.5 mm thick above stipe, 0.5 mm thick at the edge; violaceous (17F4). ODOR not distinctive, mild. TASTE not distinctive at first, later slowly becoming pungent. LAMELLAE 6–18 mm long, 2–9 mm broad; yellowish white to pale yellow when young, becoming grayish orange (5B3) from basidiospores, edges concolorous; sinuate, subdistant, sigmoid and narrow at first, becoming moderately broad, eventually broad in larger basidiomata; edges smooth. STIPE 10–61 mm long and width 2–3 mm at apex, 2–4 mm in middle, and 2–9 mm at base; rarely equal, typically clavate; ± solid and stuffed but not fragile; apex either white and glabrous or (when young) entirely covered with dark purplish violet (17 or 18F6–7) dense fibrils and punctate-squamules; when mature rimulose with dense fibrils and scattered punctae forming darker reticulate ridges around grayish violet fibrillose gaps; basal tomentum scarce to absent; basal rhizoids absent. VEIL possibly present as indicated by the fibrils on the otherwise white stipe apex and the purplish violet fibrils on the crenulate pileal edge.



PLATE 1. *Leptonia subpanniculus* (DLL10260 holotype): A. basidiomata;
B. pileus surface (left), stipe surface, and lamellae (right): Bars = 14 mm.

BASIDIOSPORES 5–7 distinct angles, at times the angles slightly more pronounced and protuberate, in profile view isodiametric to heterodiametric, on average heterodiametric, apex typically rounded-triangular, rarely truncate, frequently with a suprahilar depression just above the adaxial side of the hilar appendage; $9.2\text{--}13.4 \times 6.0\text{--}9.5 \mu\text{m}$ ($x = 11.1 \pm 0.9 \times 7.7 \pm 0.6 \mu\text{m}$; $E = 1.1\text{--}1.8$; $Q = 1.44 \pm 0.1$; $n/11 = 242$). BASIDIA 2–4-sterigmate, subclavate and somewhat tapered to the base, $31\text{--}54 \times 10\text{--}17 \mu\text{m}$ ($x = 43.3 \pm 3.9 \times 12.4 \pm 2.9 \mu\text{m}$;

E = 2.4–4.8; Q = 3.44 ± 0.6 ; n/3 = 25). LAMELLAR EDGE fertile. CHEILOCYSTIDIA absent. PLEUROCISTIDIA absent. LAMELLAR HYPHAE subparallel, $99\text{--}159 \times 6\text{--}13 \mu\text{m}$ (n/1 = 6). PILEIPELLIS from $100\text{--}300 \mu\text{m}$ thick, in young basidiomata the hyphae typically entangled along the entire surface with the terminal 2–5 cells forming a distinct trichodermium, in older basidiomata a trichodermium on the disc, forming semi-erect clusters towards the margin and forming a periclinal layer of entangled hyphae at the edge. PILEOCYSTIDIA cylindric to cylindro-clavate; typically long, $62\text{--}233 \times 9\text{--}16 \mu\text{m}$ ($x = 123.6 \pm 49.78 \times 11.1 \pm 2.1 \mu\text{m}$; E = 5.5–17.3; Q = 11 ± 3.3 ; n/2 = 15). PILEUS TRAMA hyphae subparallel in radial section, $179\text{--}298 \times 8\text{--}15 \mu\text{m}$ (n/1 = 3). STIPITIPPELLIS similar to the pileipellis along the entire length, but forming a cutis at the apex. CAULOCYSTIDIA similar to the pileocystidia, $48\text{--}204 \times 6\text{--}11 \mu\text{m}$ (n/2 = 15). STIPE TRAMAL HYPHAE subparallel, $200\text{--}367 \times 6.0\text{--}12.9 \mu\text{m}$ (n/1 = 4). OLEIFEROUS HYPHAE rare to absent in the trama. BRILLIANT GRANULES typically absent but at times very scattered in basidia. LIPOID GLOBULES absent. PIGMENTS in the suprapellis hyphae cytoplasmic and brownish to purplish, in the hyphae of the subpellis and outer pileal trama both cytoplasmic and purplish as well as encrusted and dark brownish; pigments eventually producing a purplish grey exudate in water, 10% NH_4OH , and 3% KOH. CLAMP CONNECTIONS abundant in all tissues.

ECOLOGY & DISTRIBUTION — Solitary or scattered in soft crumbly soil at the bottom of a moss-covered depression or along a path in soil protected by roots in a closed canopy forest just above the falls in Mt. Hypipamee National Park or within moss-covered trenches in open canopy in Longlands State Forest, mid to late March.

ADDITIONAL COLLECTIONS EXAMINED — AUSTRALIA. QUEENSLAND, Cook Region, Mt. Hypipamee National Park, $17^\circ 25' 35''\text{S}$ $145^\circ 29' 12''\text{E}$, 14 March 2009, DL Largent 9647; 18 March 2009, DL Largent 9668; $17^\circ 25' 35''\text{S}$ $145^\circ 29' 16''\text{E}$, 17 March 2011, DL Largent 10034; $17^\circ 25' 35''\text{S}$ $145^\circ 29' 15''\text{E}$, 25 March 2011, DL Largent 10070; $17^\circ 25' 35''\text{S}$ $145^\circ 29' 17''\text{E}$, 14 March 2012, DL Largent 10282; $17^\circ 25' 37''\text{S}$ $145^\circ 29' 15''\text{E}$, 20 March 2012, DL Largent 10306; Herberton National Park, Longlands Gap State Forest, $17^\circ 27' 07''\text{S}$ $145^\circ 28' 33''\text{E}$, 24 March 2009, DL Largent 9697; $17^\circ 27' 17''\text{S}$ $145^\circ 28' 33''\text{E}$, 1 March 2012, DL Largent 10227; 10 March 2012, DL Largent 10266; $17^\circ 27' 16''\text{S}$ $145^\circ 28' 34''\text{E}$, 14 March 2012, DL Largent 10285.

DISTINCTIVE CHARACTERS—Mycenoid to collybioid, purplish violet basidiomata with a possible veil, stipe typically developing reticulate or punctate ridges in age; basidiospores heterodiametric, averaging $11 \times 8 \mu\text{m}$, distinctly angular (occasionally with slightly protuberant angles), apex in profile view often rounded or truncate and with an adaxial depression, suprapellis with cytoplasmic brownish (occasionally purplish) pigments, and the subpellis with both cytoplasmic purplish and encrusting dark brownish pigments.



PLATE 2. *Leptonia subpanniculus* (DLL10260 holotype): A. stipitipellis longitudinal section at apex; B. pileipellis radial section near disc; C. cylindro-clavate and narrowly obclavate pileocystidia (terminal cells); D. pileipellis subpellis and outer pileal trama; encrusted and cytoplasmic pigment; and clamp; E. basidia and basidioles; F. basidiospores. Bars: A = 60 μ m; B = 50 μ m; C, E = 12 μ m; D = 15 μ m; F = 8 μ m.

COMMENTS—*L. subpanniculus* is morphologically very similar to *Entoloma panniculus* (Berk.) Sacc. from Tasmania, which differs in its deep blue coloration, farinaceous odor, oily taste, and basidiospore size ($9\text{--}12 \times 6\text{--}8 \mu\text{m}$) (Noordeloos & Gates 2012).

***Leptonia substricta* Largent & Bergemann, sp. nov.**

PLATES 3, 4

MYCOBANK MB 813880

Differs from *Entoloma strictum* by its mycenoid basidiomata with yellowish white to pale yellow adnexed lamellae, mild taste and odor, and obclavate caulocystidia.

TYPE — Australia, New South Wales, central Hunter District, Myall Lakes National Park, 32°32'30"S 152°18'39"E, in sandy soil, 10 April 2010, DL Largent 9852 (**holotype** DAR; GenBank KR869894 (mtSSU), KR869923 (LSU), KR869949 (RPB2)).

ETYMOLOGY — named for its similarity to *E. strictum*.

BASIDIOMATA mycenoid. PILEUS 10–20 mm broad, 8–15 mm high, at first evenly conic to campanulate, in age convex and obscurely umbonate, edge decurved, even when young, becoming crenulate in age; surface matted squamulose becoming matted fibrillose towards the edge; indigo-blue (18F3) to dark violet (18F6) with the squamule tips more blackish purple; opaque, neither translucent nor hygrophanous. CONTEXT 0.5–1.0 mm. above the stipe. ODOR & TASTE mild. LAMELLAE 8–9 mm long, 3–4 mm broad, adnexed, narrow to moderately wide, close to subdistant, edges eroded in age; bluish white at first, then yellowish white (4A2–3) to pale yellow (4A3), becoming orangish white (5A2–3) as spores mature; where injured becoming brownish; edges concolorous. LAMELLULAE typically in 3 (rarely 4) series, 1–2 short, 1 medium, and 1 medium long. STIPE 40–90 × 2–3 mm, equal; solid but fragile and snaps easily; surface at times longitudinally furrowed, densely appressed fibrillose-squamulose; blackish blue (19F6), squamules with purplish black punctate tips abundant at the apex, moderately abundant to scarce in the middle, and very scattered to nearly absent at the base; base with abundant to copious whitish blue tomentum, occasionally also with rhizoids projecting 10–20 mm into the soil.

BASIDIOSPORES 5–7 distinct angles, in profile subisodiametric to heterodiametric (on average heterodiametric), apex typically rounded, rarely truncate; 8–12 × 6–10 μm ($x = 10.5 \pm 0.9 \times 7.7 \pm 0.8 \mu\text{m}$; $E = 1.1\text{--}1.7$; $Q = 1.38 \pm 0.14$; $n/2 = 59$). BASIDIA 4-sterigmate, clavate, broad, 28–50 × 7–14 μm ($x = 41.1 \pm 8.5 \times 11 \pm 2.3 \mu\text{m}$; $E = 3.4\text{--}4.1$; $Q = 3.71 \pm 0.28$; $n/2 = 9$). LAMELLAR EDGE fertile. CHEILOCYSTIDIA absent. PLEUROCISTIDIA absent. LAMELLAR TRAMAL HYPHAE subparallel, short to moderately long, 34–192 × 6.5–14 μm ($x = 113 \pm 47 \times 10 \pm 2.6 \mu\text{m}$; $E = 4\text{--}19$; $Q = 11.7 \pm 5.0$; $n/2 = 9$). PILEIPELLIS at first a trichodermium over the entire surface, 150–300 μm deep, composed of 3–5 cells with the pileocystidia erect and the subterminal cells longitudinally entangled, remaining so with age on central 2/3 but becoming semi-erect towards the margin and nearly prostrate at the edge. PILEOCYSTIDIA versiform (clavate to cylindro-clavate, subulate to obclavate), 46–100 × 7–19.5 μm ($x = 74.6 \pm 15.9 \times 12.6 \pm 4.1 \mu\text{m}$; $E = 3\text{--}11$; $Q = 6.6 \pm 2.6$; $n/2 = 21$). PILEAL TRAMAL HYPHAE subparallel 58–226 × 4–22 μm ($x = 119 \pm 63 \times 12.1 \pm 6.3 \mu\text{m}$; $E = 4\text{--}52$; $Q = 14.2 \pm 16.8$; $n/2 = 7$). STIPITPELLIS composed of clusters of erect to semi-erect 2–5 celled-hyphae separated by a 2–3-celled prostrate cutis; terminal cells differentiated. CAULOCYSTIDIA similar in shape but often much



PLATE 3. *Leptonia substricta* (DLL 9852 holotype): basidiomata. Bar = 10 mm.

longer than the pileocystidia, $48\text{--}156 \times 6\text{--}13\text{ }\mu\text{m}$ ($x = 81.8 \pm 35.8 \times 9.2 \pm 1.7\text{ }\mu\text{m}$; $E = 4\text{--}19$; $Q = 9.2 \pm 4.4$; $n/2 = 13$). STIPE TRAMAL HYPHAE subparallel, $200\text{--}367 \times 6.0\text{--}13\text{ }\mu\text{m}$; $n/2 = 9$). OLEIFEROUS HYPHAE rare in the lamellar trama, absent elsewhere. BRILLIANT GRANULES absent. LIPOID GLOBULES absent. PIGMENTS in pilei-/stipitipellis brownish blue, cytoplasmic; in pileus subpellis dark brown, encrusting. CLAMP CONNECTIONS in all tissues.

ECOLOGY & DISTRIBUTION—Barrington Tops National Park: scattered, humicolous, subtropical gallery rainforest, mid-March; Mungo Brush, Myall

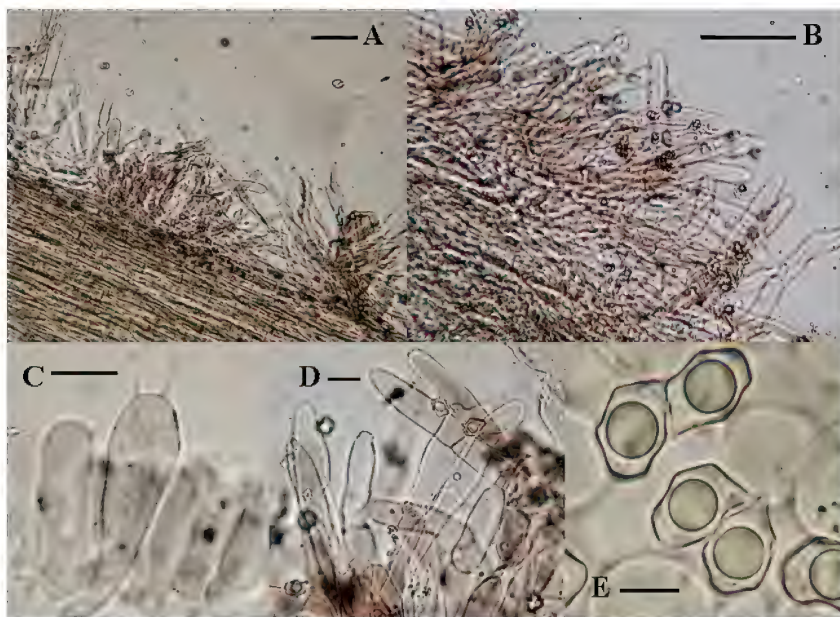


PLATE 4. *Leptonia substricta* (DLL 9852 holotype): A. stipitipellis longitudinal section at apex; B. pileipellis radial section near disc; C. basidia and basidioles; D. broadly obclavate pileocystidia and cytoplasmic pigment; E. basidiospores. Bars: A = 125 μ m; B = 50 μ m; C = 9 μ m; D, E = 7 μ m.

Lakes National Park: exposed in sandy soil along the trail in a littoral rainforest, early April.

ADDITIONAL COLLECTION EXAMINED —AUSTRALIA. NEW SOUTH WALES, central Hunter District. Barrington Tops National Park, Williams Day Use Area, 16 March 2009, S Moore 1.

DISTINCTIVE CHARACTERS—Basidiomata mycenoid; pileus conic to campanulate, appressed squamulose then matted squamulose to matted fibrillose towards edge, indigo-blue to dark violet (18F6) with the squamule-tips more blackish purple; stipe long and narrow, very fragile, blackish blue and densely appressed fibrillose but with abundant to scarce purplish black-tipped squamules; lamellar edge fertile; pileocystidia subulate to obclavate; Pilei-/stipitipellis with brownish blue cytoplasmic pigments, pileus subpellis with encrusting pigments; taste and odor mild.

COMMENTS—*Leptonia substricta* morphologically resembles *Entoloma strictum* G. Stev. (Horak 2008), which differs by its collybioid basidiomata, (arcuate)

decurrent pink lamellae, farinaceous taste and odor, and clavate or vesiculose caulocystidia. *Entoloma cedretorum* (Romagn. & Rioussel) Noordel., from southern France and Morocco, also close to *L. substricta*, differs in its white lamellae, entirely wooly-tomentose stipe, and intracellular pigment.

***Leptonia sabulosa* Largent & Bergemann, sp. nov.**

PLATES 5, 6

MYCOBANK MB 813883

Differs from *Entoloma endotum* by its yellowish young lamellae, punctuate squamulose stipe, mild taste, sandy habitat, and smaller basidiospores.

TYPE — Australia, New South Wales, central Hunter District, Tomaree National Park, 32°45'26"S 152°08'03"E, in sandy soil, 23 April 2012, DL Largent 10427 (holotype DAR, GenBank KR869896 (mtSSU), KR869925 (LSU)).

ETYMOLOGY — derived from the Latin *sabulosus* referring to the fruiting in sandy soil.

BASIDIOMATA tricholomatoid. PILEUS 27–64 mm broad, 7–12 mm high; when young convex to broadly convex (not umbonate), expanding to plane and broadly umbonate; at first uniformly appressed squamulose with flat overlapping squamules that later collapse to form a uniformly velvet-like surface; dull (neither translucent nor hygrophanous), center dark blackish blue with a dark violet tint (18–19F5–7), somewhat lighter (18–19E5–7) towards the margin, still lighter (18E5–8) on the very edge; outer margin incurved at first,



PLATE 5. *Leptonia sabulosa* (DLL10427 holotype): basidiomata. Bar = 25 mm.

becoming decurved, then plane and eventually uplifted, the edge extending beyond the lamellae by 1 mm. CONTEXT 2–5 mm thick above the stipe, solid, watery grey. ODOR somewhat pungent. TASTE mild. LAMELLAE 13–25 mm long, 5–10 mm broad; sinuate, close to subdistant, moderately wide to wide, edges smooth; initially yellowish white to orange-white (4–5A2), becoming orange-white to grayish orange (5A–B2–3) from spores with concolorous edges. LAMELLULAE in 3 tiers between lamellae, 2 short and 1 longer. STIPE 37–75 mm long, 5–9 mm thick at apex and middle, 7–12 mm thick at base; stuffed at first becoming hollow at the apex; overall densely fibrillose with the fibrils splitting laterally and with abundant black-edged punctae from apex to base (at the very base rare in some), at the base tomentum abundant, forming a broad pad; blackish-blue (19F7–8) when young (becoming more greyish violet (18–19E5) as the fibrils split apart), dark blue (19E6) at the base; basal tomentum staining orangish (4A2–3).

BASIDIOSPORES 5–7 distinct angles (at times slightly more pronounced and protuberate), in profile subisodiametric to heterodiametric (on average decidedly heterodiametric, only rarely isodiametric), the apex typically rounded, rarely truncate; $8\text{--}13 \times 6\text{--}9 \mu\text{m}$ ($x = 10.4 \pm 0.8 \times 7.0 \pm 0.6 \mu\text{m}$; $E = 1.1\text{--}1.8$; $Q = 1.5 \pm 0.1$; $n/3 = 100$). BASIDIA 4-sterigmate, clavate, $37\text{--}54 \times 6.5\text{--}13.5 \mu\text{m}$ ($x = 45.1 \pm 5.1 \times 11.0 \pm 1.4 \mu\text{m}$; $E = 3\text{--}8$; $Q = 4.2 \pm 0.9$; $n/3 = 27$). LAMELLAR EDGE fertile. CHEILOCYSTIDIA absent. PLEUROCYSTIDIA absent. LAMELLAR TRAMAL HYPHAE subparallel, $46\text{--}135 \times 5\text{--}23 \mu\text{m}$ ($x = 83.0 \pm 29.1 \times 12.8 \pm 6.8 \mu\text{m}$; $E = 2\text{--}17$; $Q = 8.3 \pm 4.8$; $n/1 = 12$). PILEIPELLIS 122–321 μm deep, a $\pm$ erect entangled layer of 3–6-celled hyphae; PILEOCYSTIDIA cylindro-clavate to broadly cylindro-clavate and often cystidiiform, $43\text{--}128 \times 5\text{--}17 \mu\text{m}$ ($x = 80.5 \pm 21.9 \times 11.4 \pm 3.0 \mu\text{m}$; $E = 2\text{--}16$; $Q = 7.8 \pm 3.6$; $n/2 = 30$). PILEAL TRAMAL HYPHAE entangled, 13–28 μm wide ($n/1 = 7$). STIPITIPELLIS a layer of hyphae 4–6-cells deep, composed of abundant clusters of semi-erect to erect anticlinal hyphae interspersed among periclinal hyphae. CAULOCYSTIDIA cylindro-clavate, $51\text{--}86 \times 7\text{--}13 \mu\text{m}$ ($x = 74.5 \pm 13.5 \times 10.0 \pm 2.6 \mu\text{m}$; $E = 5\text{--}12.5$; $Q = 7.9 \pm 2.6$; $n/2 = 10$). STIPE TRAMAL HYPHAE 8–28 μm diam ($n/1 = 7$). OLEIFEROUS HYPHAE absent. BRILLIANT GRANULES scattered in the basidia. LIPOID BODIES absent. PIGMENTATION purplish blue, cytoplasmic in the pileipellis and stipitipellis. CLAMP CONNECTIONS abundant in all tissues.

ECOLOGY & DISTRIBUTION—Scattered in sandy soil and leaves in the forest between the beach and mangrove stands in northeastern Queensland, late March; also beneath grass trees (species of *Xanthorrhoea* Sm.) and cycads (species of *Macrozamia* Miq.) in central New South Wales, April.



PLATE 6. *Leptonia sabulosa* (DLL10427 holotype): A. pileipellis radial section near disc; B. stipitipellis longitudinal section at apex; C. cylindro-clavate pileocystidia (terminal cells); D. basidiospores; E. basidia and basidioles. Bars: A, B = 50 μ m; C = 80 μ m; D = 7 μ m; E = 11 μ m.

ADDITIONAL COLLECTIONS EXAMINED —AUSTRALIA. QUEENSLAND, Cook Region, Yorkies Knob 16°49'01"S 145°43'50"E, 21 March 2011, DL Largent 10058. NEW SOUTH WALES, central Hunter District, Wyrribalong National Park, 33°17'40"S 151°32'38.20"E, P O'Sullivan NW 010; Tomaree National Park, 32°45'26"S 152°08'02"E, 24 April 2012, DL Largent 10445.

DISTINCTIVE CHARACTERS—Basidiomata tricholomatoid; pileus dark blackish blue with a dark violet tint; appressed squamulose pileus becoming velvety in age; young yellowish tinged lamellae developing a decidedly grayish orange cast when mature; cheilocystidia absent; basidiospores with slightly protuberant angles; odor pungent, taste mild.

COMMENTS—*Entoloma endotum* Noordel. & G.M. Gates from Tasmania is distinguished from *L. sabulosa* by its creamy pink young lamellae, fibrillose-floccose or girdled stipe, a salty or bitter to acrid taste, and somewhat larger basidiospores (10.5–12.7 $\times$ 7.9–8.5 μ m). *Entoloma eugenei* differs by its sterile lamellar edges, pure white lamellae, blackish blue colors (violet tinted in the

pileus), cylindrical, lageniform, or irregularly shaped cheilocystidia, and strangulated pilei- and caulocystidia.

Leptonia newlingii Largent & Bergemann, sp. nov.

PLATES 7, 8

MYCOBANK MB 813886

Differs from *Entoloma indoviolaceum* by its medium to large basidiomata, fertile lamellar edges, slightly shorter basidiospores, and the cytoplasmic pigments in both pilei- and stipitipellis.

TYPE — Australia, Queensland, Cook Region, Yorkies Knob Beach Forest, 16°48'59"S 145°43'49"E, in sandy soil, 11 March 2011, DL Largent 10004 (holotype BRI; isotype CNS; GenBank KR869897 (mtSSU), KR869926 (LSU)).

ETYMOLOGY — named for Peter Newling, who discovered it.

PILEUS 11–51 × 5–18 mm, initially convex to campanulate (not umbonate), then plane to uplifted and broadly umbonate; entirely punctate tomentose from blunt erect pointed scales that flatten to form a uniformly appressed velvety surface; dull, opaque but not translucent; blackish blue (19F5) when young, becoming slightly lighter (19F4) on the disc and somewhat lighter (19E4) elsewhere; the outer margin incurved then decurved to plane, the edge in age crenulate to eroded, extending past lamellae by 0.5–1 mm. CONTEXT 0.5–2 mm thick above the stipe, violaceous to bluish white. ODOR mild. TASTE mild (occasionally somewhat pungent to farinaceous). LAMELLAE 7–25 × 2.5–8 mm; adnexed to nearly adnate; at first close and narrow, then subdistant and moderately broad to broad with smooth then eroded edges; initially pale yellow (2A3) to yellowish white (4A2), becoming pale orange (5A3) as spores mature, with concolorous edges. LAMELLULAE in 1 or 3 series, 1 medium or 2 short (≤ 3 mm) and 1 medium (≤ 11 mm). STIPE 42–73 mm long, the 2–7 mm diam. apex expanding to 7–15 mm base; at first apex punctate from erect squamules, elsewhere densely fibrillose-squamulose, later remaining punctate at the apex but becoming rimose and longitudinally reticulate-ridged elsewhere with the ridges composed of fibrils interspersed with appressed squamules; at first blackish blue (20F6), with the ridges lightening slightly (20D4), and then finally dark blue (19E4); context stuffed then hollow, more or less yellowish orange with a hint of orange (4–5A3); stipe base with scarce to moderately abundant white (at times yellowish white, 2A2) tomentum and abundant yellowish white rhizoids spreading into the soil.

BASIDIOSPORES 5–7 distinct to rounded angles, isodiametric to heterodiametric (on average heterodiametric) in profile view, apex at first somewhat rounded and $\pm$ triangular (in some becoming truncate and in a few with the angles slightly protuberant); $8.5\text{--}12.5 \times 6.5\text{--}9\text{ }\mu\text{m}$ ($x = 10.4 \pm 0.7 \times 7.7 \pm 0.6\text{ }\mu\text{m}$; $E = 1\text{--}1.6$; $Q = 1.4 \pm 0.1$; $n/3 = 132$). BASIDIA 4-sterigmate; clavate,



PLATE 7 *Leptonia newlingii* (DLL10004 holotype): A. basidiomata (field); B. basidiomata (lab).
Bars = 30 mm.

tapered towards the base, $33\text{--}59 \times 10\text{--}14.5 \mu\text{m}$ ($x = 46.0 \pm 6.6 \times 11.9 \pm 1.1 \mu\text{m}$, $E = 3\text{--}4.6$, $Q = 3.8 \pm 0.5$, $n/2 = 17$). LAMELLAR EDGE fertile. CHEILOCYSTIDIA absent. PLEUROCYSTIDIA absent. LAMELLAR TRAMAL HYPHAE subparallel, moderately long and broad: $53\text{--}180 \times 5\text{--}15 \mu\text{m}$ ($x = 94.5 \pm 31.3 \times 8.7 \pm 2.9 \mu\text{m}$;

E = 5–20; Q = 11.5 ± 4.0 ; n/2 = 16). PILEIPELLIS 90–250 μm (n/1 = 3) deep, composed of trichodermial tufts comprising 3–4 celled hyphae, loosely entangled, and erect terminal cells. PILEOCYSTIDIA cylindro-clavate to clavate, $45\text{--}157 \times 6.5\text{--}12.5 \mu\text{m}$ ($x = 95.8 \pm 27.9 \times 9.6 \pm 1.7 \mu\text{m}$; E = 5–17; Q = 10.2 ± 3.2 ; n/2 = 21). PILEAL TRAMAL HYPHAE subparallel, entangled, short and cylindric to cylindro-clavate; $80\text{--}240 \times 9\text{--}22 \mu\text{m}$ ($x = 152.6 \pm 64.9 \times 15.5 \pm 4.0 \mu\text{m}$; E = 5–15; Q = 9.9 ± 3.6 ; n/2 = 8). STIPITIPPELLIS composed of loosely entangled hyphae with clavate terminal cells. CAULOCYSTIDIA similar to pileocystidia but shorter and more slender, $50\text{--}123 \times 5\text{--}8 \mu\text{m}$ ($x = 82.3 \pm 20.7 \times 6.9 \pm 0.9 \mu\text{m}$; E = 7–19; Q = 12.2 ± 3.2 ; n/3 = 21). STIPE TRAMAL HYPHAE subparallel and entangled. OLEIFEROUS HYPHAE absent. BRILLIANT GRANULES scattered in the basidia. LIPOID GLOBULES absent. PIGMENTS in pileipellis and stipitipellis bluish and cytoplasmic, soluble in 3% KOH, brownish pigments and encrusted pigments absent. CLAMP CONNECTIONS abundant in all tissues.

ECOLOGY & DISTRIBUTION—Scattered in sandy soil and leaves, in forest between the beach and mangrove stands or in soil under logs in vine forests, in northeastern Queensland, mid-January to late March.

ADDITIONAL COLLECTIONS EXAMINED —AUSTRALIA. QUEENSLAND, Yorkies Knob Beach Forest, $16^{\circ}48'51''\text{S } 145^{\circ}43'47''\text{E}$, 11m, 9 January 2011, P. Newling PN14b; $16^{\circ}48'49''\text{S } 145^{\circ}43'44''\text{E}$, 14 March 2011, DL Largent 10026, $16^{\circ}46'00''\text{S } 145^{\circ}43'49''\text{E}$, 22 March 2012, DL Largent 10313.

DISTINCTIVE CHARACTERS—Basidiomata at first collybioid but soon naucorioid to nearly trichomatoid; pileus at first blackish blue and entirely tomentose or erect squamulose, then dark blue and tomentose on disc and appressed squamulose elsewhere; lamellae yellowish white; stipe fleshy, similar in color to pileus, with ridges and bands of squamules; basal tomentum moderate to abundant; stipe base with rhizoids penetrating the substrate.

COMMENTS—*Leptonia newlingii* and *E. indoviolaceum* (from Kerala state in India; Manimohan et al. 2006) are closely related phylogenetically (BS = 71, FIG. 1). Morphologically, *E. indoviolaceum* is distinguished from *L. newlingii* by its small to medium basidiomata, sterile lamellar edges, longer (11–14 μm) basidiospores, and encrusting pigments in the stipitipellis.

Entoloma panniculus from Tasmania can be distinguished from *L. newlingii* by its smaller basidiomata, farinaceous odor and taste, and encrusting pigments in the stipitipellis.

Since 2009, numerous *L. newlingii* populations have been observed in the fall season, occurring abundantly in sandy soils in coastal vine forests just north of Cairns in northeastern Queensland (P. Newling, pers. obs.).



PLATE 8 *Leptonia newlingii* (DLL10004 holotype): A. pileipellis; B. pileocystidia; C. basidiospores; D. caulocystidia; E. basidium; F. pileocystidia. Bars: A = 170 μ m; B, D, F = 10 μ m; C = 8 μ m; E = 12 μ m.



PLATE 9 *Leptonia ambigua* (DLL10130 holotype): A. basidiomata;
B. basidiospores; C. submoniliform pileipellis. Bars: A = 16 mm; B = 7.5 µm; C = 10 µm.

Leptonia ambigua Largent, Mycotaxon 125: 18 (2013).

PLATE 9

COMMENTS—Our analysis supports the placement of *L. ambigua* within a sub-clade that includes *Entoloma euchroum* (FIG. 1). *Leptonia ambigua* can be distinguished from the other species within this sub-clade by its isodiametric to subisodiametric basidiospores with 6–7(–8) angles and a pileipellis of submoniliform clampless hyphae (PLATE 9C). For a discussion of morphological similarities and differences with other entolomatoid species see the description of *L. ambigua* in Largent et al. (2013b).

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***Dictyoaquaphila appendiculata* gen. & sp. nov. from submerged wood from Brazil**

JOSIANE SANTANA MONTEIRO¹, LUCAS BARBOSA CONCEIÇÃO²,
MARCOS FÁBIO OLIVEIRA MARQUES², LUÍS FERNANDO PASCHOLATI GUSMÃO^{1*}
& RAFAEL F. CASTAÑEDA-RUIZ²

¹ Universidade Estadual de Feira de Santana,

Avenida Transnordestina, s/n, Novo Horizonte, 44036-900, Feira de Santana, Brazil

² Universidade do Estado da Bahia,

Rodovia Lomanto Jr., BR 407 Km 127, 48970-000, Senhor do Bonfim, Brazil

³ Instituto de Investigaciones Fundamentales en Agricultura ‘Alejandro de Humboldt’ (INIFAT),

Calle 1 Esq. 2, C.P. 17200, Santiago de Las Vegas, C. Habana, Cuba

* CORRESPONDENCE TO: lgusmao@uefs.br

ABSTRACT — *Dictyoaquaphila appendiculata*, collected from submerged wood in Brazil, is described and illustrated as a new genus and species. It is distinguished by distinct, erect, septate, subhyaline to very pale brown conidiophores, mono- and polyblastic conidiogenous cells, and fusiform, elliptical to shortly clavate, brown separating cells. The conidia are dictyoseptate, broad ellipsoidal-fusiform to prolate subglobose, dark brown, usually with 1–3 divergent, narrow cylindrical, hyaline appendages at the apical cell, and sometimes with 1–2(–3), narrow cylindrical, hyaline appendages arising from the basal cell.

KEY WORDS — freshwater fungi, neotropic, taxonomy

Introduction

The diversity of conidial fungi in the Brazilian Amazon Forest has received little attention, especially in freshwater habitats. During a survey of hyphomycetes associated with submerged plant material in streams from Pará state, a conspicuous fungus was collected. This fungus shows remarkable differences from all previously described genera (Seifert et al. 2011) and is described here as new.

Materials & method

Samples of submerged litter were placed in plastic bags for transport to the laboratory, where they were placed in Petri dish moist chambers and stored at 25°C for 30 days in a 170 L polystyrene box with 200 mL sterile water plus 2 mL glycerol (Castañeda-Ruiz 2005). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol) and measurements were made at a $\times 1000$ magnification. Micrographs were obtained with an Olympus BX51 microscope equipped with bright field and Nomarski interference optics. The specimens including holotype are deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Dictyoaquaphila J.S. Monteiro, L.B. Conc., M.F.O. Marques, Gusmão & R.F. Castañeda **gen. nov.**

MYCOBANK MB 813243

Differs from *Dictyodesmium* by its effuse colonies, polyblastic and swollen conidiogenous cells, fusiform separating cells, and dictyoseptate conidia with several cylindrical filamentous hyaline appendages at one or both end cells.

TYPE SPECIES: *Dictyoaquaphila appendiculata* J.S. Monteiro et al.

ETYMOLOGY: Greek, *dictyo-*, referring to the transverse, longitudinal, and oblique conidial septa; Latin, *-aqua-* meaning water; and Greek, *-phila* meaning loving.

Conidial fungi. COLONIES on the natural substratum effuse, brown or dark brown. CONIDIOPHORES distinct, single, unbranched, septate, subhyaline to pale brown. CONIDIOGENOUS CELLS mono- and polyblastic, integrated or discrete, determinate or indeterminate with sympodial extensions. CONIDIOGENOUS LOCI flatted or slightly broad denticulate, subhyaline or pale pigmented. SEPARATING CELLS swollen, fusiform, ellipsoidal or shortly clavate, brown or dark brown. Conidial secession schizolytic. CONIDIA solitary, dictyoseptate, broad ellipsoid-fusiform to prolate subglobose, brown to dark brown, cuneate to subulate or subconical at the end cells, with 0–3 divergent filiform or narrow cylindrical, hyaline appendages at the ends.

Dictyoaquaphila appendiculata J.S. Monteiro, L.B. Conc., M.F.O. Marques, Gusmão & R.F. Castañeda **sp. nov.**

FIG 1–3

MYCOBANK MB 813244

Differs from all *Dictyodesmium* spp. by its effuse and mono- or polyblastic colonies, sometimes with sympodial extensions, and its indeterminate conidiogenous cells from which arise swollen, fusiform, or shortly clavate separating cells and dictyoseptate conidia, appendiculate at the end cells.

TYPE: Brazil, Pará State: Santa Bárbara, Parque Ecológico de Gumma, 1°13'S 48°17'W, on decaying submerged wood in a river, 29.VII.2012, coll. J.S. Monteiro (**Holotype**: HUEFS 216013).

ETYMOLOGY: Latin, *appendiculata*, referring to the small conidial appendages.

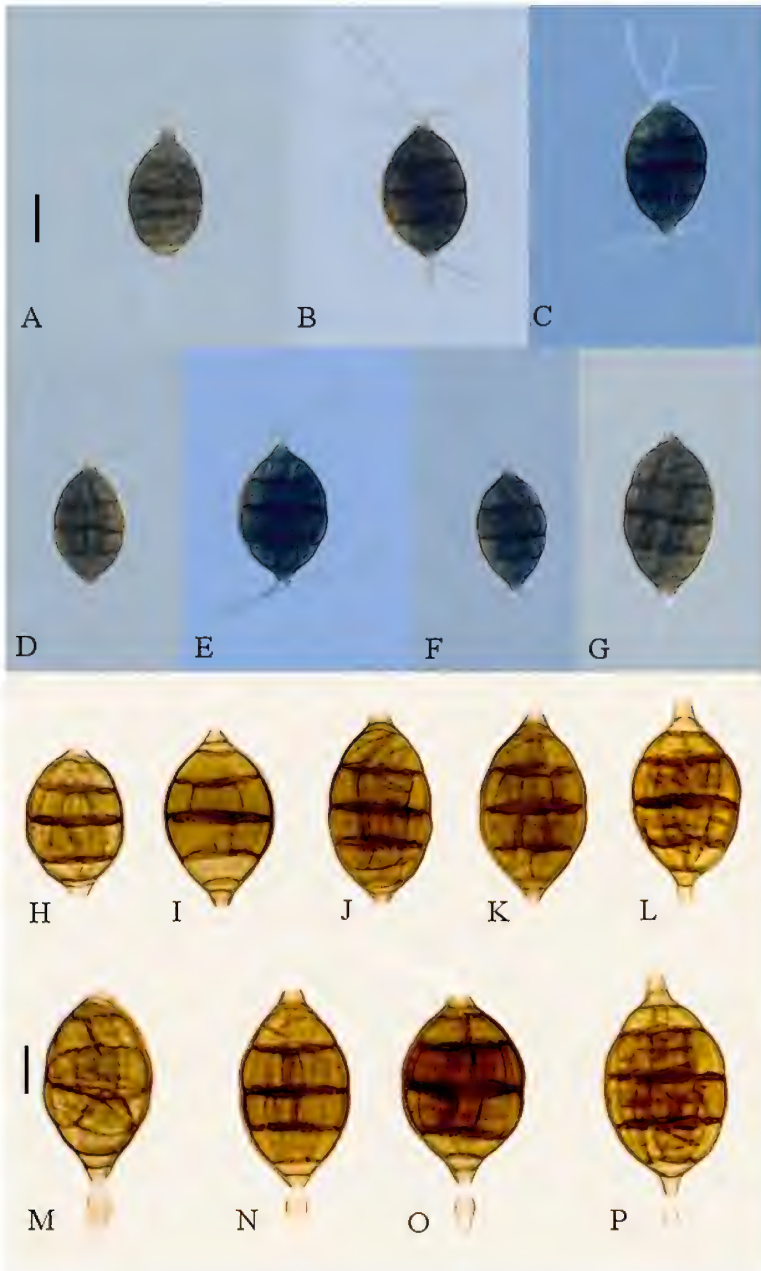


FIG. 1. *Dictyoaquaphila appendiculata* (ex holotype HUEFS 216013).
Conidia. Bar = 10 μ m.

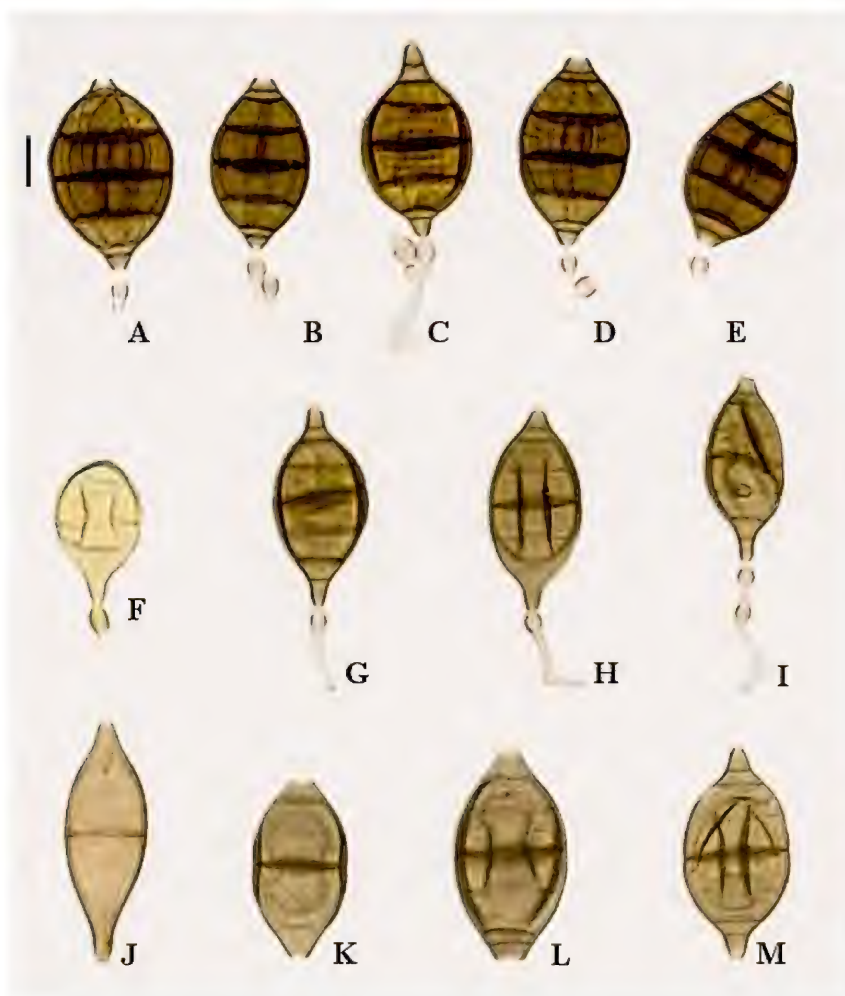


FIG. 2. *Dictyoaquaphila appendiculata* (ex holotype HUEFS 216013).
A–I. conidiogenous cells, separating cells, and conidia; J–M. conidia. Bar = 10 μm.

COLONIES on the natural substratum effuse, dark brown to black. Mycelium mostly immersed. CONIDIOPHORES distinct, single or moderately branched, erect, bent or flexuous, subhyaline or very pale brown, 0–3-septate, $20\text{--}40 \times 3\text{--}5 \mu\text{m}$, smooth. CONIDIOGENOUS CELLS mono- and polyblastic, integrated, sometimes discrete, determinate or indeterminate with sympodial extensions, terminal and intercalary, subhyaline to very pale brown, $3\text{--}5 \times 1.5\text{--}3 \mu\text{m}$.

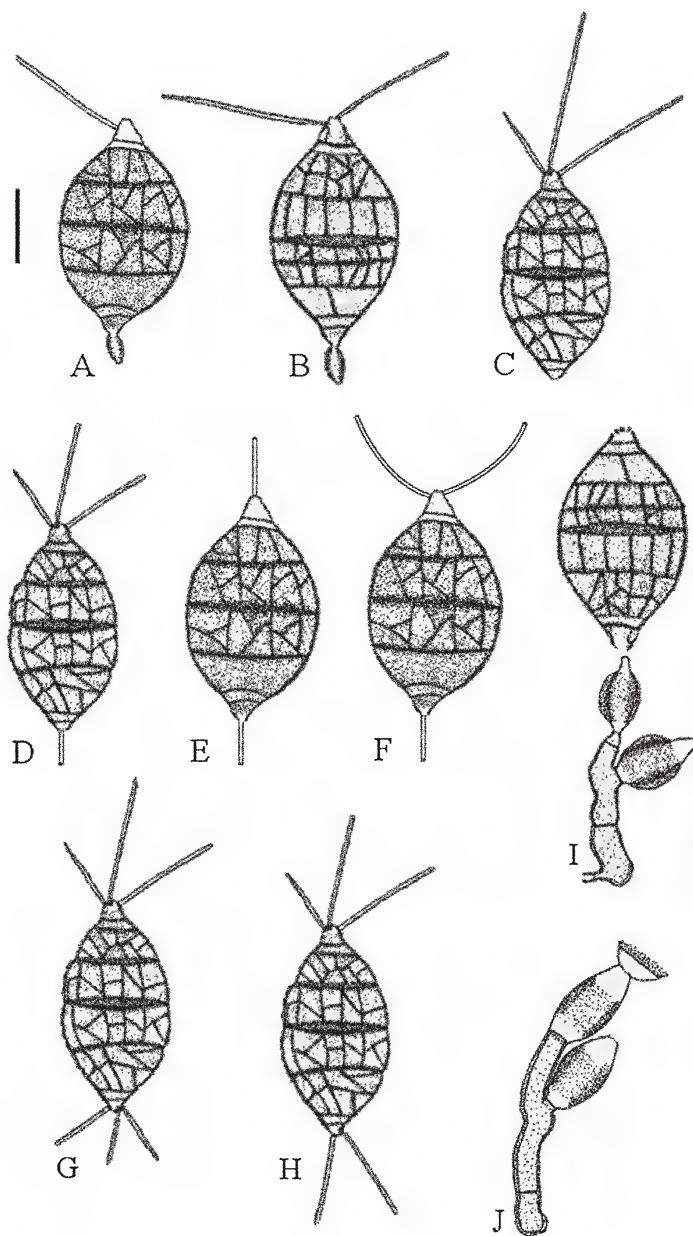


FIG. 3. *Dictyoaquaphila appendiculata* (ex holotype HUEFS 216013).
A–H. conidia; I–J. conidiophores, conidiogenous cells and separating cells. Bar = 10 μ m.

SEPARATING CELLS swollen, fusiform, ellipsoidal or shortly clavate, brown or dark brown at the margin, pale brown at the middle, subhyaline at the ends, sometimes in a short chains with a secondary cell, $7\text{--}10 \times 3.5\text{--}5\text{ }\mu\text{m}$. Conidial secession schizolytic. CONIDIA solitary, acrogenous, dictyoseptate, broad ellipsoid-fusiform to prolate subglobose, $37\text{--}45 \times 17\text{--}27\text{ }\mu\text{m}$, brown to dark brown, black at the transverse septa, and subhyaline or hyaline at the cuneate to subulate or subconical at the end cells, mostly with 0–3 divergent, filiform or narrow cylindrical, hyaline, smooth apical appendages, $20\text{--}42 \times 1\text{--}1.5\text{ }\mu\text{m}$ and sometimes 0–2(–3) divergent, filiform or narrow cylindrical, hyaline, smooth basal appendages, $20\text{--}35 \times 1\text{--}1.5\text{ }\mu\text{m}$.

ADDITIONAL SPECIMEN EXAMINED: BRAZIL, BAHIA STATE: Pindobaçu, Serra da Fumaça, on decaying submerged wood in stream, 7.XII.2012, L.B. Conceição (HUEFS 216020).

COMMENTS: *Dictyoaquaphila* is superficially similar to *Dictyodesmium* S. Hughes, *Dictyorostrella* U. Braun, *Muiaria* Thaxt., and *Xenostigmina* Crous, all of which have short distinct conidiophores and a blastic conidial ontogeny but which lack separating cells and filiform appendages (Seifert et al 2011). Filiform appendages, which are widely distributed in the aero-aquatic and freshwater hyphomycete ecological group, probably function in anchoring the conidia to substrates (Cai et al. 2006, Ingold 1975, Seifert et al. 2011). The function of separating cells in freshwater hyphomycetes has not been documented, although Kendrick (2003) did cover some hyphomycete genera with biconic conidia attached at the separating cells.

Acknowledgments

The authors express their sincere gratitude to Dr. De-Wei Li and Prof. Bryce Kendrick for their critical review of the manuscript. JSM is grateful to the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” for Pos-doctoral fellowship (proc. 071/2012). LFPG is grateful to the CNPq (Proc. 303062/2014-2). RFCR is grateful to Cuban Ministry of Agriculture and “Programa de Salud Animal y Vegetal”, project P131LH003033 Cuban for facilities. We acknowledge the assistance provided by Dr. P.M. Kirk and Drs. V. Robert and G. Stegehuis through the Index Fungorum and MycoBank websites. Dr. Lorelei Norvell’s editorial and Dr. Shaun Pennycook’s nomenclatural reviews are greatly appreciated.

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***Anacoronospora diversiseptata* gen. & sp. nov. from Brazil**

JOSIANE SANTANA MONTEIRO¹, LUÍS FERNANDO PASCHOLATI GUSMÃO^{1*} &
RAFAEL F. CASTAÑEDA-RUIZ²

¹ Universidade Estadual de Feira de Santana,

Avenida Transnordestina, s/n, Novo Horizonte, 44036-900, Feira de Santana, Brazil

² Instituto de Investigaciones Fundamentales en Agricultura 'Alejandro de Humboldt' (INIFAT),

Calle 1 Esq. 2, C.P. 17200, Santiago de Las Vegas, C. Habana, Cuba

* CORRESPONDENCE TO: lgusmao@uefs.br

ABSTRACT — The hyphomycete *Anacoronospora diversiseptata*, collected from decaying leaves from Caatinga biome, Brazil, is described and illustrated as a new genus and species. It is distinguished by mono- and polyblastic conidiogenous cells with successive sympodial rectilinear or geniculate extension (sometimes delimited by one septum and followed by enteroblastic percurrent extension), which process is repeated several times. The conidia are oval to broad ellipsoidal, golden brown, and with a distoseptate main body, a subcylindrical or doliiform basal cell with 0–4 mammillate protuberances, and a crown-shaped euseptate apex with 3–4 divergent cylindrical branches.

KEY WORDS — asexual fungi, neotropic, taxonomy

Introduction

During a survey of hyphomycetes associated with plant litter from the semi-arid region of Bahia State, a fungus was collected that differs remarkably from all previously described genera (Seifert et al. 2011) and which we describe here as new. Caatinga ecosystems are unique to Brazil and, with only about 1% of the area under protection, are undoubtedly threatened. The type locality is a small remaining fragment of natural or semi-natural vegetation surrounded by farming and other developments. The discovery of this new genus here (and of others already collected by the authors, but as yet undescribed) suggests that the Caatinga may be as rich in fungi as in the many endemic animals and plants.

Materials & methods

Samples of litter (leaves and wood) were collected in paper bags for transport to the laboratory, where they were placed in Petri dish moist chambers and stored at 25°C for 30 days in a 170 L polystyrene box with 200 mL sterile water plus 2 mL glycerol (Castañeda-Ruiz 2005). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol) and measurements were made at a magnification of $\times 1000$. Micrographs were obtained with an Olympus BX51 microscope equipped with bright field and Nomarski interference optics. The type specimens are deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Anacoronospora J.S. Monteiro, Gusmão & R.F. Castañeda, **gen. nov.**

MYCOBANK MB 813219

Differs from *Coronospora* by its successive enteroblastic percurrent regeneration after each holoblastic sympodial extension of the cicatrized conidiogenous cells and its distoseptate and euseptate conidia with small basal protuberances.

TYPE SPECIES: *Anacoronospora diversiseptata* J.S. Monteiro et al.

ETYMOLOGY: Greek, *ana-*, meaning upwards; Latin *coronospora*, referring to the hyphomycete genus *Coronospora*.

Conidial fungi. COLONIES on the natural substratum effuse, brown or dark brown. Mycelium partly superficial and immersed. CONIDIOPHORES distinct, single, unbranched, septate, brown or dark brown. CONIDIOGENOUS CELLS mono- and polyblastic, integrated, with enteroblastic percurrent regenerations and sympodial extensions. CONIDIOGENOUS LOCI evident, flattened, thick and black. Conidial secession schizolytic. CONIDIA solitary, at first acrogenous, later acropleurogenous, ovoid, ellipsoidal, with several short protuberances on the basal cell, distoseptate in the main body, crown-shaped at the apex, with several divergent, aseptate or euseptate branches, brown to dark brown.

Anacoronospora diversiseptata J.S. Monteiro, Gusmão & R.F. Castañeda **sp. nov.**

FIGS 1–3

MYCOBANK MB 813220

Differs from all *Coronospora* spp. by its successive enteroblastic percurrent regenerations after each holoblastic sympodial extension of the conidiogenous cells and solitary disto- and euseptate, conidia with several short protuberances at the basal cell.

TYPE: Brazil, Bahia State: Abaíra, Distrito de Catolés, Mata do Coqueiro, 13°14'S 41°43'W, on decaying leaves of unidentified plant, 11.I.2015, coll. J.S. Monteiro (Holotype: HUEFS 215981).

ETYMOLOGY: Latin, *diversi-*, meaning turned different ways, diverse, contrary, different; *septata*, referring to the septa.

COLONIES on the natural substratum effuse, hairy, amphigenous, brown to dark brown. Mycelium mostly immersed. CONIDIOPHORES distinct, single,



FIG. 1. *Anacoronospora diversiseptata* (ex holotype HUEFS 215981): A–C, conidiophores, conidiogenous cells, and conidium; D, E, conidiogenous cells and conidium; F, conidiophore and conidiogenous cells. Bars = 10 μ m.

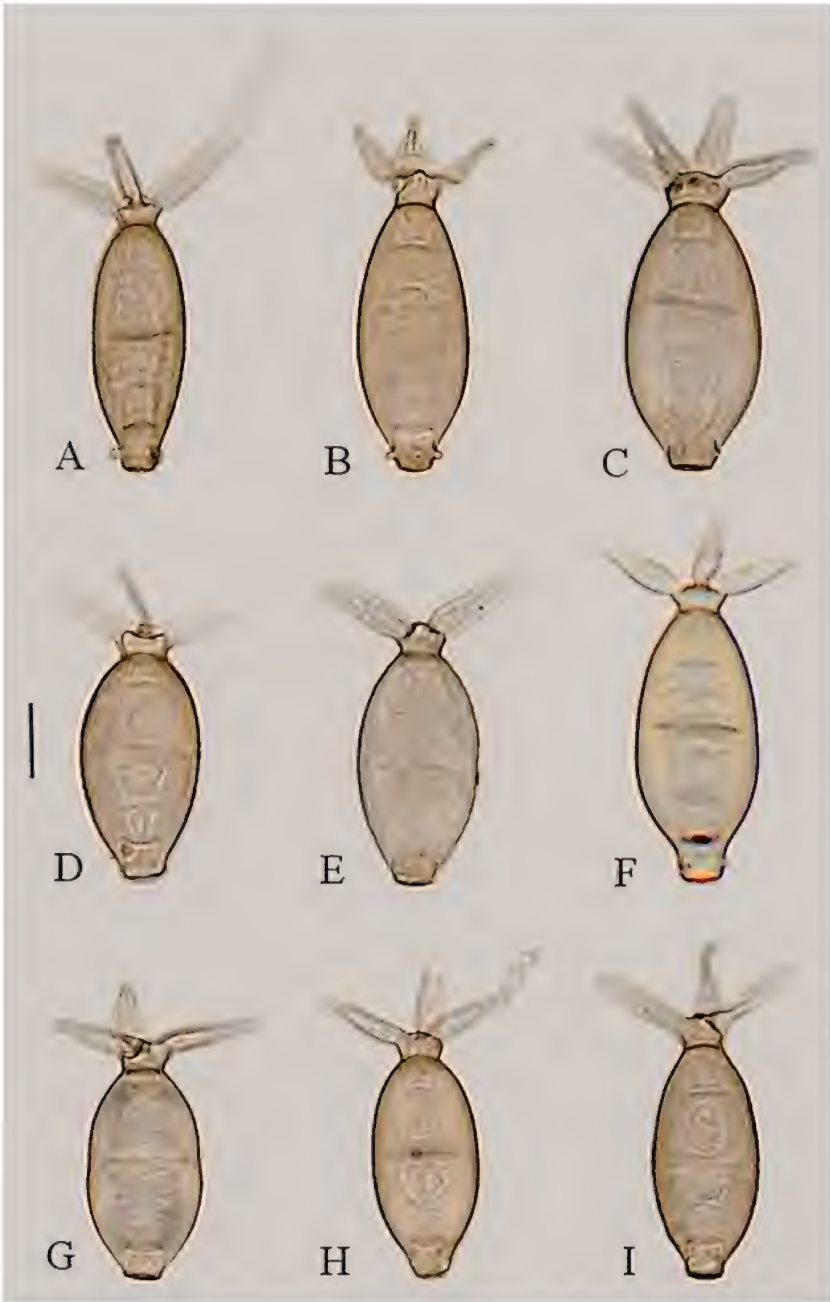


FIG. 2. *Anacoronospora diversiseptata* (ex holotype HUEFS 215981): Conidia. Bar = 10 μ m.

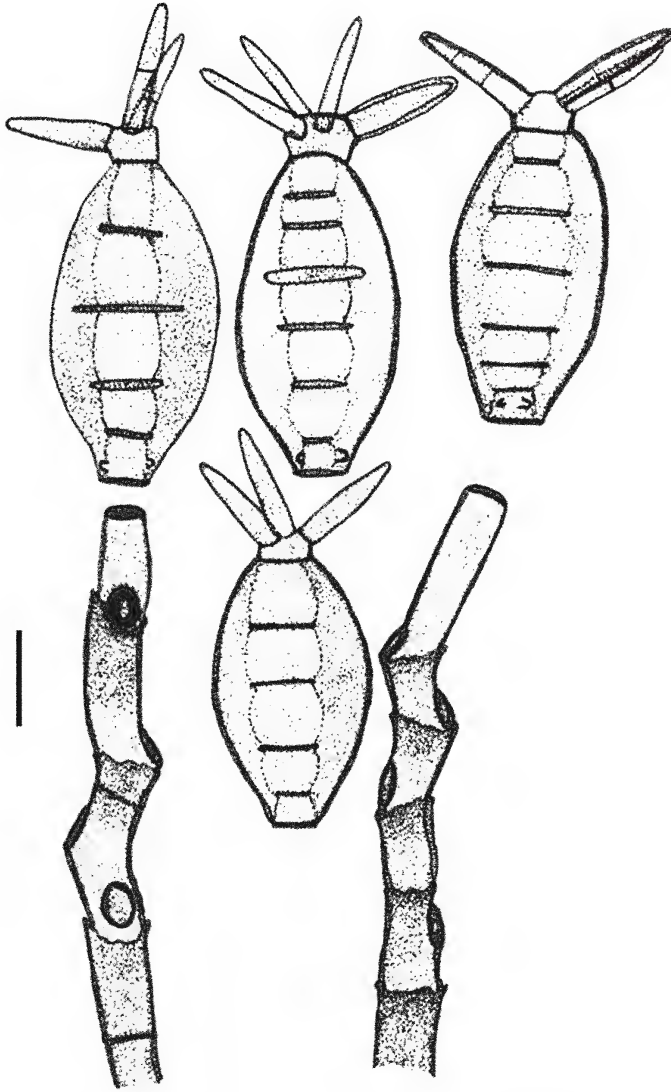


FIG. 3. *Anacoronospora diversiseptata* (ex holotype HUEFS 215981):
Conidiogenous cells and conidia. Bar = 10 μ m.

erect, straight or slightly geniculate toward the apex, with up to 7 percurrent regenerations, 5–10-septate, 70–150 $\times$ 5–7 μ m, dark brown below, brown to pale brown toward the apex, somewhat fasciate after each enteroblastic regeneration,

smooth. CONIDIOGENOUS CELLS mono- and polyblastic, integrated, with enteroblastic percurrent regenerations and sympodial extensions, terminal at first with a single terminal conidiogenous locus, then indeterminate, with successive sympodial rectilinear or geniculate extension, sometimes delimited by one septum and followed by enteroblastic percurrent extension, being the same process repeated several times, producing terminal and intercalary conidiogenous loci. Conidiogenous loci cicatrized, flat, dark brown or black. Conidial secession schizolytic. CONIDIA solitary, acropleurogenous, $30\text{--}45 \times 14\text{--}19 \mu\text{m}$, ovoid to broad ellipsoidal, 4–5(–7)-distoseptate at the main body, golden brown, with subcylindrical, truncate, $5\text{--}6 \mu\text{m}$ wide, cicatrized basal cell, with 0–4 nipple-shaped protuberances radially arranged and crown-shaped, euseptate at the apex, with a cuneiform or doliiform apical cell from which arise 3–4 divergent, cylindrical, obtuse at the tip, 0–2-euseptate, pale golden brown appendages, $10\text{--}20 \times 3\text{--}4 \mu\text{m}$.

COMMENTS — The ontogenetic pattern in *Anacoronospora* can be classified using the scheme for conidial development in Kirk et al. (2008). Conidial initiation is holoblastic, delimitation by one septum, secession schizolytic, maturation by diffuse wall-building, percurrent enteroblastic conidiogenous cell extension, followed by further conidial initiation by replacement of apical wall-building; melanized, with each successive conidium seceding before the next percurrent elongation of the conidiogenous cell, but sometimes holoblastic sympodial extension also occurs, producing two or more conidia. The combination of sympodial and enteroblastic percurrent extension and regeneration with flattened, thick, darkened conidiogenous loci in *Anacoronospora* separates it from *Coronospora* M.B. Ellis, in which cicatrized loci are produced after sympodial extensions of the polyblastic cicatrized conidiogenous cells disposed in geniculate conidiophores (Ellis 1971, 1976, Matsushima 1975, 1985, Zhang & Zhang 2004). Two described *Coronospora* species (FIG. 4), *C. novae-zelandiae* Matsush. and *C. uniseptata* Matsush., have denticulate, non-cicatrized conidiogenous loci, characters that deviate somewhat from those found in *Coronospora dendrocalami* M.B. Ellis (the type species) and *C. pallescens* Meng Zhang & T.Y. Zhang. Phylogenetic analysis is needed to elucidate the taxonomic position of *C. novae-zelandiae* and *C. uniseptata*.

Acknowledgments

The authors express their sincere gratitude to Dr. De-Wei Li and Prof Bryce Kendrick for their critical review of the manuscript. The authors thank the “Programa de Pesquisa em Biodiversidade do Semiárido (PPBio Semiárido - CNPq/MCTI) for supporting this study. LFPG is grateful to the CNPq (Proc. 303062/2014-2). JSM is grateful to the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” for

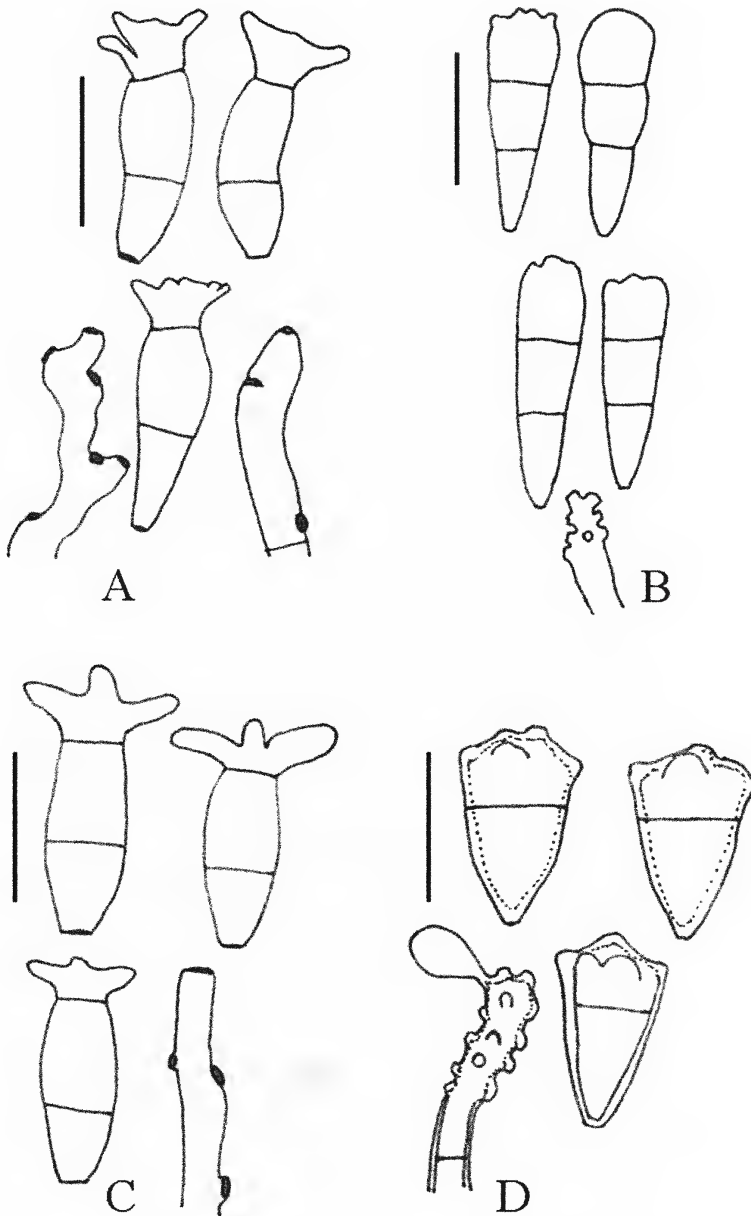


FIG. 4. Representative conidia of *Coronospora* spp. (redrawn from literature): A. *C. dendrocalami*; B. *C. novae-zelandiae*; C. *C. pallescens*; D. *C. uniseptata*. Bars = 20 μ m.

Post-doctoral fellowship (proc. 071/2012). RFCR is grateful to Cuban Ministry of Agriculture and “Programa de Salud Animal y Vegetal”, project P131LH003033 Cuban for facilities. We acknowledge the assistance provided by Dr. P.M. Kirk and Drs. V. Robert and G. Stegehuis through the Index Fungorum and MycoBank websites. Dr. Lorelei Norvell’s editorial and Dr. Shaun Pennycook’s nomenclatural reviews are greatly appreciated.

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Two new polypore species from the southwestern USA: *Fomitiporia fissurata* and *F. deserticola*

JOSEF VLASÁK^{1*} & JOSEF VLASÁK JR.

¹*Biological Centre of the Academy of Sciences of the Czech Republic,
Branišovská 31, CZ-370 05 České Budějovice, The Czech Republic*

* CORRESPONDENCE TO: vlasak@umbr.cas.cz

ABSTRACT — Based on molecular evidence and morphological studies, two new *Fomitiporia* species are described from southwestern USA. *Fomitiporia fissurata* sp. nov., on living *Quercus*, was previously misdetermined as *F. robusta* and is the only oak-inhabiting *Fomitiporia* species in the region. *Fomitiporia deserticola* sp. nov. grows on cacti and desert shrubs in Arizona and was previously misdetermined as *F. texana*, which is a species found only on juniper.

KEY WORDS — *Basidiomycota*, *Hymenochaetales*, *Hymenochaetaceae*, molecular taxonomy

Introduction

Fomitiporia Murrill (*Hymenochaetales*, *Basidiomycota*) forms a monophyletic clade within *Hymenochaetaceae* and is characterized by a perennial habit, dextrinoid and cyanophilous basidiospores, and absence of setae in most species (Dai et al. 2008; Dai 2010). Over the years, the interpretation of *Fomitiporia* in the USA has changed and evolved. Murrill (1907, 1908) described many American species that were not accepted by subsequent mycologists. During the most of the 20th century, the European species *F. robusta* (P. Karst.) Fiasson & Niemelä, *F. punctata* (P. Karst.) Murrill, and *F. hartigii* (Allesch. & Schnabl) Fiasson & Niemelä were reported throughout the United States. Only *F. texana* (Murrill) Nuss and *F. sonoreae* (Gilb.) Y.C. Dai from southwestern USA were recognized as endemic American species (Gilbertson & Ryvarden 1987; Dai et al. 2001).

Recently, the presence of *F. robusta* and *F. punctata* in southern, western, and eastern USA has been questioned (Fischer & Binder 2004; Vlasák & Kout

2011a) whereas other species, described by Murrill, have been confirmed. For example, recognition of resupinate species *Fomitiporia dryophila* Murrill, *F. langloisii* Murrill, and *F. maxonii* Murrill has been supported by molecular phylogenetics (Decock et al. 2007). Pileate species *F. bakeri* (Murrill) Vlasák & Kout growing on birch and *F. calkinsii* (Murrill) Vlasák & Kout growing on oak from the eastern USA have also been confirmed (Vlasák & Kout 2011a). In addition, two new species—*F. cupressicola* Amalfi et al. growing on *Cupressus arizonica* in northern Mexico and *F. polymorpha* M. Fisch. on *Salix* from the USA Pacific Coast—have been described (Fischer & Binder 2004; Amalfi et al. 2012). In these studies, researchers noted that DNA sequence data, ecological preferences, and biogeographical data were important in species characterization because distinguishing microscopic features were absent.

Overholts (1953) realized that a peculiar form of *Fomes robustus* [$\equiv$ *Fomitiporia robusta*], which was “less developed and nodulose-sessile,” occurred on oaks in the western USA. Vlasák & Kout (2011a), who obtained a sample of this taxon from California (voucher JV 0407/16-J), discovered that its ITS rDNA sequence differed from all previously described *Fomitiporia* species. Amalfi et al. (2012) obtained the same ITS sequence from a collection from New Mexico designated *Fomitiporia* PS1 (phylogenetic species 1). We have since collected many specimens of this oak-restricted and relatively common fungus from southern California and Arizona, and describe it below as a new species, *F. fissurata*.

Davidson & Mielke (1947) identified a different taxon found on cacti and other desert plants in Arizona as a variety of *Fomes robustus*. Based on studies of pure cultures, they concluded it was not conspecific with *F. texana*, which is known from the same region but grows on juniper. *Pyropolyporus texanus* [$\equiv$ *Fomitiporia texana*] was considered conspecific with *Fomes robustus* by Lowe (1957) and Lowe & Gilbertson (1961) but were later accepted as distinct species, with the taxon growing on desert plants treated as conspecific with *F. texana* (Gilbertson & Canfield 1972; Gilbertson & Ryvarden 1987). We collected *Fomitiporia* specimens from both juniper and desert plants in Texas and Arizona and conclude that they represent separate species. We describe the taxon growing on desert plants as the new species *Fomitiporia deserticola*.

Materials & methods

Samples were collected in July 2004, spring and fall 2012, May 2013, and March 2014 at various localities in California and Arizona. The specimens were dried and microscopically examined in Melzer's reagent (IKI) and 5% KOH. All collected specimens are deposited in herbarium of Josef Vlasak (JV); types deposited in Prague Museum herbarium, Czech Republic (PRM). DNA isolation, nuclear ribosomal DNA

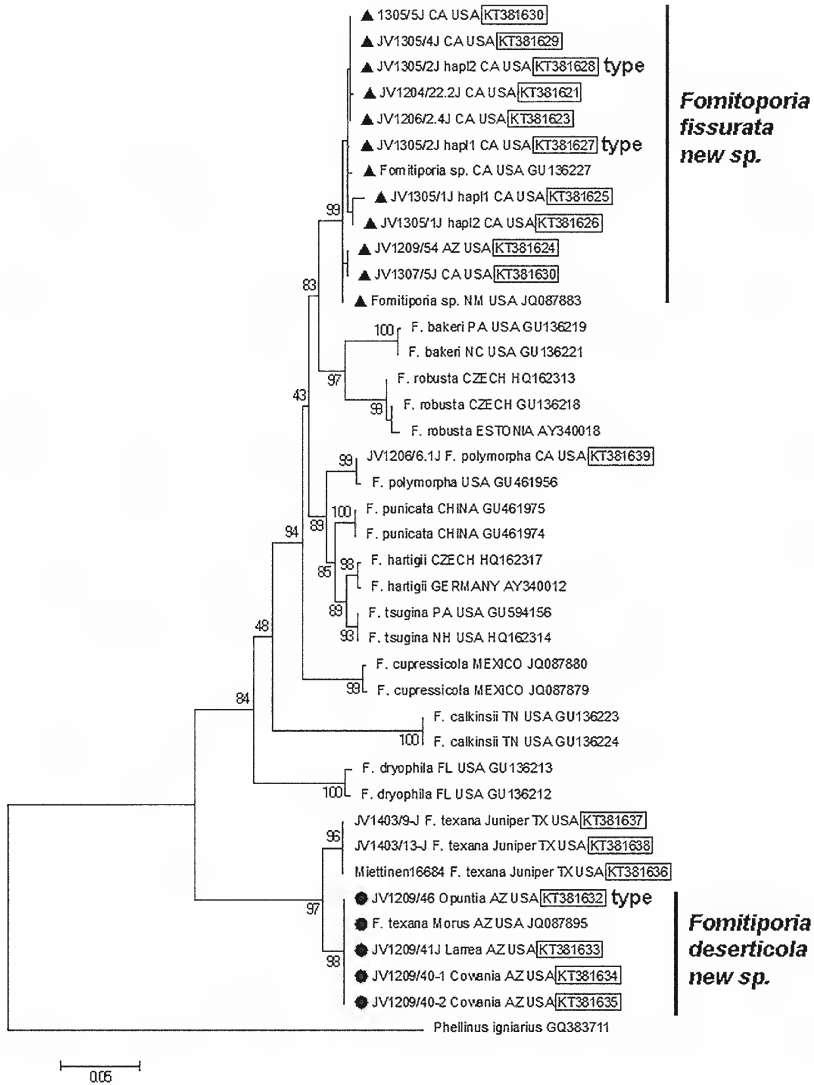


FIG. 1: Phylogenetic relationships of 36 *Fomitiporia* specimens inferred from ITS rRNA sequences. *Phellinus igniarius* was used as outgroup. Topology from maximum likelihood (ML) analysis. Support values along branches from ML bootstrap (500 replicates). Branch lengths are drawn proportional to the number of substitutions per site. Triangles and black circles indicate specimens of new species. GenBank numbers are presented at the end of taxon labels with new sequences boxed.

ITS region sequencing, and comparative phylogenetic analyses methods follow Vlasák & Kout (2011b). The evolutionary history was inferred by using the Maximum Likelihood method; the analyses were conducted using MEGA 6 (Tamura et al. 2013).

Results

Phylogenetic analysis

Twenty ITS sequences of *Fomitiporia* specimens from California and Arizona were generated for this study. A complete *Fomitiporia* ITS dataset, which included 23 *Fomitiporia* sequences retrieved from GenBank and represented all pileate *Fomitiporia* species occurring in the USA, produced an alignment with 833 characters, of which 180 were parsimony informative. Phylogenetic analyses strongly support all pileate *Fomitiporia* specimens collected on oak in California and Arizona within the clade of the new species *F. fissurata* (FIG. 1). Species in this clade are sister to the European species *F. robusta* and the American species *F. bakeri*, as expected from their morphological characters. *Fomitiporia* specimens from various desert plants in Arizona (including cacti) comprise another strongly supported clade, representing the new species *F. deserticola*, which is sister to the juniper-dwelling *F. texana* (FIG. 1).

Taxonomy

Fomitiporia fissurata Vlasák, sp. nov.

FIGS 2, 3

MYCOBANK MB 813966

Differs from *Fomitiporia robusta* by its broader, rounded pileal margin, pileal surface with deep arboriform fissures even in juvenile basidiomata, smaller basidiospores, and a different ITS rDNA sequence.

TYPE: USA. California: Cleveland National Forest, Mt. Laguna, *Quercus* sp. (*Fagaceae*), 18 May 2013, J. Vlasák Jr. (Holotype, PRM 922626; isotype, JV1305/2-J; GenBank KT381627, KT381628).

ETYMOLOGY: *fissurata* (Lat.): referring to striking fissures on pileal surface of the fungus.

BASIDIOMES perennial, pileate, arising from a black, cracked, crust-like outgrowth on the substrate, unguulate, up to 18 × 10 × 12 cm; margin 3–6 cm broad, rounded; upper surface gray to blackish, often with white spore deposits, at first glabrous and with a few wide sulcate zones, soon indurated to 1 cm deep and cracking up irregularly, creating deep, often arboriform fissures; pore surface yellowish brown; pores circular, 4–6 per mm, with thin to thick, entire dissepiments; context yellowish-brown, shining at first, then woody hard, up to 5 cm thick, with distinct white radial veins in older parts resulting in a marmorate appearance of the context close to the substrate attachment; tube layers distinct, light brown, tubes becoming white within from hyphae growing in the tubes, woody, each layer up to 1 cm thick.



FIG. 2: *Fomitiporia fissurata* specimen: PRM 922626, holotype (JV1305/2-J, isotype)



FIG. 3: *Fomitiporia fissurata* specimen: JV1209/54 (split collection: PRM 922627)

HYPHAL SYSTEM dimitic, skeletal hyphae brownish, thick-walled, 2–4 µm diam., generative hyphae hyaline, thin-walled, simple septate, 2–4 µm diam. SETAE absent. BASIDIA broadly ellipsoid, 4-sterigmate, 12–15 × 7–9 µm, simple septate at the base. BASIDIOSPORES subglobose, hyaline, smooth, thick-walled, strongly dextrinoid, 6–7 × 5–6(–6.5) µm. White rot on living *Quercus*.

DISTRIBUTION & ECOLOGY. Common on living *Quercus* spp. in the US southwest (California, Arizona, New Mexico); causing a white rot. No other pileate *Quercus*-dwelling *Fomitiporia* species is known in the region.

ADDITIONAL SPECIMENS EXAMINED: USA. CALIFORNIA: Santa Cruz, Henry Covell Redwoods State Park, *Quercus*, Jul 2004, J. Vlasák Jr. (JV0407/16J; GenBank GU136227, GU136228); Thousand Oaks, Malibu Creek State Park, *Quercus*, 22 Apr 2012, J. Vlasák Jr. (JV1204/22.2J; GenBank KT381621, KT381622); Saratoga, Castle Rock State Park, *Quercus*, 2 Apr 2006, J. Vlasák Jr. (JV1206/2.4J; GenBank KT381623); Cleveland National Forest, Pine Valley County Park, *Quercus*, 18 May 2013, J. Vlasák Jr. (JV1305/1J; GenBank KT381625, KT381626); Escondido, Mt. Palomar, *Quercus*, 23 May 2013, J. Vlasák Jr. (JV1305/4J; GenBank KT381629); Palm Springs, Mt. San Jacinto, *Quercus*, 25 May 2013, J. Vlasák Jr. (JV1305/5J; GenBank KT381630); ARIZONA: Santa Rita Mts., Madera Canyon, *Quercus*, 2 Sep 2012, J. Vlasák (JV1209/19J; Chiricahua Mts., Chiricahua Nat. Monument Visitor Center, *Quercus*, 5 Sep 2012, J. Vlasák (JV1209/54, PRM 922627; GenBank KT381624); Cochise Stronghold National Monument, *Quercus*, 17 Jul 2013, J. Vlasák Jr. (JV1307/5J; GenBank KT381631).

COMMENTS. *Fomitiporia robusta*, which occurs in Europe, differs from *F. fissurata* by its pileal surface mostly covered with green algae and becoming cracked only in old basidiomes. The cracks do not form arboriform fissures. In addition, a black crust does not develop around the *F. robusta* basidiome, which has a narrower rounded margin compared to *F. fissurata*. Finally, *F. robusta* produces larger basidiospores (6–8.5 × 5.5–7 µm). *Fomitiporia bakeri* also resembles *F. fissurata* but is found only on birch in the eastern and central USA. *Fomitiporia calkinsii*, which grows on oaks in the southeastern USA, has a relatively sharp margin, a more thinly hardened crust that is rarely or inconspicuously cracked, and a darker brown context and tube layers. *Fomitiporia polymorpha*, growing primarily on willows in the western USA, develops small, bulbous basidiomes, and *F. hartigii* and *F. tsugina* are known from gymnosperms.

***Fomitiporia deserticola* Vlasák, sp. nov.**

Figs 4–6(top)

MYCOBANK MB 813967

Differs from *Fomitiporia texana* by its angular, thin-walled pores, a different ITS rDNA sequence, and smaller basidiospores.

TYPE: USA. Arizona: Tanque Verde, *Opuntia versicolor* Engelm. ex Toumey (Cactaceae), 4 Sep 2012, J. Vlasák 1209/46 (Holotype, PRM 934073; isotype, JV1209/46; GenBank KT381632).

ETYMOLOGY: *deserticola* (Lat.): referring to growth on desert plant substrates.



FIG. 4: *Fomitiporia deserticola* specimens:
JV1209/46, isotype (left); PRM 934073, holotype (right).



FIG. 5: *Fomitiporia deserticola* specimen: JV1209/40.

BASIDIOMES perennial, pileate, distinctly ungulate, small to medium-sized, usually 2–4 cm in diam., but sometimes up to 8 × 8 × 8 cm; upper surface at first dark brown, tomentose, soon becoming blackened and deeply rimose, usually with white spore deposits and cracks often partially filled by current year context tissue; margin rounded, yellowish brown, tomentose; pore surface pale brown; pores angular, somewhat irregular, 4–6 per mm, with thin to thick, entire dissepiments; context woody hard, brown, up to 1 cm thick; tube layers distinct, pale brown, becoming stuffed with light-colored mycelium, each layer up to 1 cm thick.

HYPHAL SYSTEM dimitic, skeletal hyphae brownish, thick-walled, 2–4 µm diam, generative hyphae hyaline, thin-walled, simple septate, 2–4 µm diam. **CYSTIDIOLES** abundant, with an inflated base up to 8 µm diam and a slender neck, 10–30 µm long and about 2 µm thick. **SETAE** rare, similar to cystidioles but thick-walled. **BASIDIA** subglobose, 4-sterigmate, 9–12 × 8–10 µm, simple septate at the base. **BASIDIOSPORES** subglobose, hyaline, smooth, thick-walled, strongly dextrinoid, 6–7.5 × 5.5–7 µm.

DISTRIBUTION & ECOLOGY. On living cacti and desert shrubs in Arizona, causing a white rot.

ADDITIONAL SPECIMENS EXAMINED:

Fomitiporia deserticola: USA, ARIZONA: Tanque Verde, *Cowania stansburiana* Torr. (*Rosaceae*), 4 Sep 2012, J. Vlasák Jr. (JV 1209/40, GenBank KT381634, KT381635); *Larrea tridentata* (Sessé & Moc. ex DC.) Coville (*Zygophyllaceae*), 4 Sep 2012, J. Vlasák Jr. (JV1209/41J; GenBank KT381633); J. Vlasák Jr. (JV1209/42J).

Fomitiporia texana: USA, TEXAS: Austin, *Juniperus ashei* J. Buchholz (*Cupressaceae*), 15 Aug 2013, O. Miettinen 16684 (JV1308/15; GenBank KT381636); Balcones Canyonlands National Wildlife Ref., *Juniperus* sp., 21 Mar 2014, J. Vlasák Jr. (JV1403/9-J; GenBank KT381637); Pedernales Falls State Park, *Juniperus* sp., 22 Mar 2014, J. Vlasák Jr. (JV1403/13-J; GenBank KT381638).

COMMENTS. *Fomitiporia texana*, which occurs in the same region as *F. deserticola*, can be distinguished by round thick-walled pores (FIG. 6, bottom), cracks on pileal surface not filled with context tissue from current year, larger basidiospores (mostly 8 µm diam), and host specific on juniper. Davidson & Mielke (1947) have also shown that culture characteristics of the cactus and juniper taxa are different.

Gilbertson & Lindsey (1975) cited setae as characteristic of *F. texana*, although setae were mentioned neither in the original description (Murrill 1908) nor in the description of *Fomes robustus* on cacti (Davidson & Mielke 1947). Gilbertson & Ryvarden (1987) suggested that these structures represent just cystidioles with thickened walls, and we agree with their interpretation.



FIG. 6: Comparison of pores of *Fomitiporia deserticola* and *F. texana*.

Setae are uncommon; as they have been observed in about half of the specimens of both *F. texana* and *F. deserticola*, their value for species differentiation is limited.

Macroscopic features key to pileate *Fomitiporia* species occurring in the USA

1	Species with detached upper margin on otherwise resupinate basidiocarp	2
1	Distinctly pileate species, with mostly large, sessile or ungulate pilei	4
2	On <i>Tsuga</i>	<i>F. tsugina</i>
2	On dicotyledonous hosts	3
3	On <i>Dodonea viscosa</i> in a small area in Arizona	<i>F. sonorae</i>
3	On <i>Quercus</i> in southern USA from Florida to Texas	<i>F. dryophila</i>
4	On <i>Betula</i> in northeastern and midwestern USA	<i>F. bakeri</i>
4	On other substrates	5
5	On coniferous hosts	6
5	On dicotyledonous hosts	7
6	On <i>Juniperus</i> in southern Texas and Arizona	<i>F. texana</i>
6	On living <i>Cupressus</i> in Mexico and southwestern USA	<i>F. cupressicola</i>
7	On desert shrubs and cacti in southern Arizona	<i>F. deserticola</i>
7	On <i>Quercus</i>	8
8	In southeastern USA, margin of typical pilei 0.5–1 cm thick, pileus crust not at all or indistinctly cracked	<i>F. calkinsii</i>
8	In southwestern USA, pileal margin rounded and 2–5 cm thick, crust deeply, irregularly cracked in old and juvenile pilei	<i>F. fissurata</i>

Discussion

With the addition of two new *Fomitiporia* species from the western USA, there are now nine North American pileate species. We do not include *F. robusta* nor *F. hartigii* in our key, because neither Eurasian species occurs in North America (Vlasák & Kout 2011a; Brazee 2013). Our DNA sequence data results support the conclusions by Amalfi et al. (2012) and others that geography, habitat, and host relationships are important factors in *Fomitiporia* speciation. Accordingly, a key based on morphology, host and distribution only (see above) can be used successfully to identify pileate species of *Fomitiporia* in North America. Nevertheless, molecular methods are necessary to delineate cryptic species.

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First report of *Singerocybe* in Thailand

JATURONG KUMLA¹, NAKARIN SUWANNARACH¹, SANTHITI VADTHANARAT¹,
OLIVIER RASPÉ^{2,3} & SAISAMORN LUMYONG^{1*}

¹Department of Biology, Faculty of Science, Chiang Mai University,
Chiang Mai, 50200, Thailand

²Botanic Garden Meise, Nieuwelaan 38, 1860 Meise, Belgium

³Fédération Wallonie-Bruxelles, Service Général de l'Enseignement universitaire
et de la Recherche scientifique, Rue A. Lavallée 1, 1080 Brussels, Belgium

* CORRESPONDENCE TO: saisamorn.l@cmu.ac.th

ABSTRACT — Specimens collected in Thailand have been identified as *Singerocybe alboinfundibuliformis*, based on morphology and ITS molecular analysis. A description and illustration are provided. This is the first record of the genus from Thailand.

KEY WORDS — Agaricales, Tricholomataceae, basidiomycete, gill fungus, taxonomy

Introduction

Harmaja (1988) erected *Singerocybe* (Agaricales, Tricholomataceae) with *S. viscida* Harmaja as the type species. This genus is distributed worldwide in temperate and tropical regions. *Singerocybe* species are saprotrophic and usually grow on soils rich in humus, dead wood, or rotting leaves (Peck 1873, Harmaja 1988, Takahashi 2000, Seok et al. 2009, Qin et al. 2014). Although no *Singerocybe* species has been reported from Thailand (Chandrasrikul et al. 2011), during a taxonomic survey of macrofungi collected in northern Thailand, we found specimens that corresponded to the description of *Singerocybe alboinfundibuliformis*, a species previously reported from China, Korea, and Japan (Takahashi 2000, Seok et al. 2009, Qin et al. 2014). Here, we describe and illustrate the morphological characters of the Thai material and provide results from ITS sequence analysis.

Materials & methods

Morphology studies

Basidiocarps were collected from Doi Suthep-Pui National Park, Chiang Mai Province, Thailand in 2015 and wrapped in aluminum foil or kept in plastic specimen boxes for transport to the laboratory where notes on macromorphological features and photographs were taken within 24 h. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C; sections of dried material were mounted in 95% ethanol and rehydrated in distilled water, 3% KOH, or Melzer's reagent for microscopical examination; at least 50 measurements were made of each structure. The collections are deposited at the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of three specimens was extracted from fresh tissue using CTAB according to Kumla et al. (2012). The internal transcribed spacer (ITS) region of ribosomal RNA gene was amplified by polymerase chain reaction (PCR) with ITS4 and ITS5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. Negative controls lacking fungal DNA were run to check for reagent contamination. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was performed by 1ST Base Company (Selangor, Malaysia) using the ITS4 and ITS5 PCR primers. Sequences were used to query GenBank database via BLAST (<http://blast.ddbj.nig.ac.jp/top-e.html>).

Results

Taxonomic description

Singerocybe alboinfundibuliformis (Seok, Yang S. Kim, K.M. Park,
W.G. Kim, K.H. Yoo & I.C. Park) Zhu L. Yang, J. Qin & Har. Takah.,
Mycologia 106: 1022 (2014).

PLATE 1

Pileus 20–50 mm in diameter, infundibuliform, hollow down to the base of stipe, smooth, white (4A1) to yellowish-white (4A2), margin involute when young and incurved in age. Lamellae decurrent, ≤1.5 mm wide, white (4A1) when young, becoming yellowish-white (4A2) to orange-white (5A2) in age. Stipe central, 20–45 × 3–10 mm, cylindrical or tapering towards the base, smooth, similar in color to the pileus. Odor strong. Pileipellis composed of irregularly parallel hyphae that are 2–5 µm wide, septate, and interspersed with vesicles (20–45 × 3–10 µm); vesicles also found at the stipe apex. Clamp connections present. Basidia 22.0–33.0 × 4.5–6.0 µm, clavate, 4-spored, with basal clamp connections and long sterigmata (≤5 µm). Basidiospores

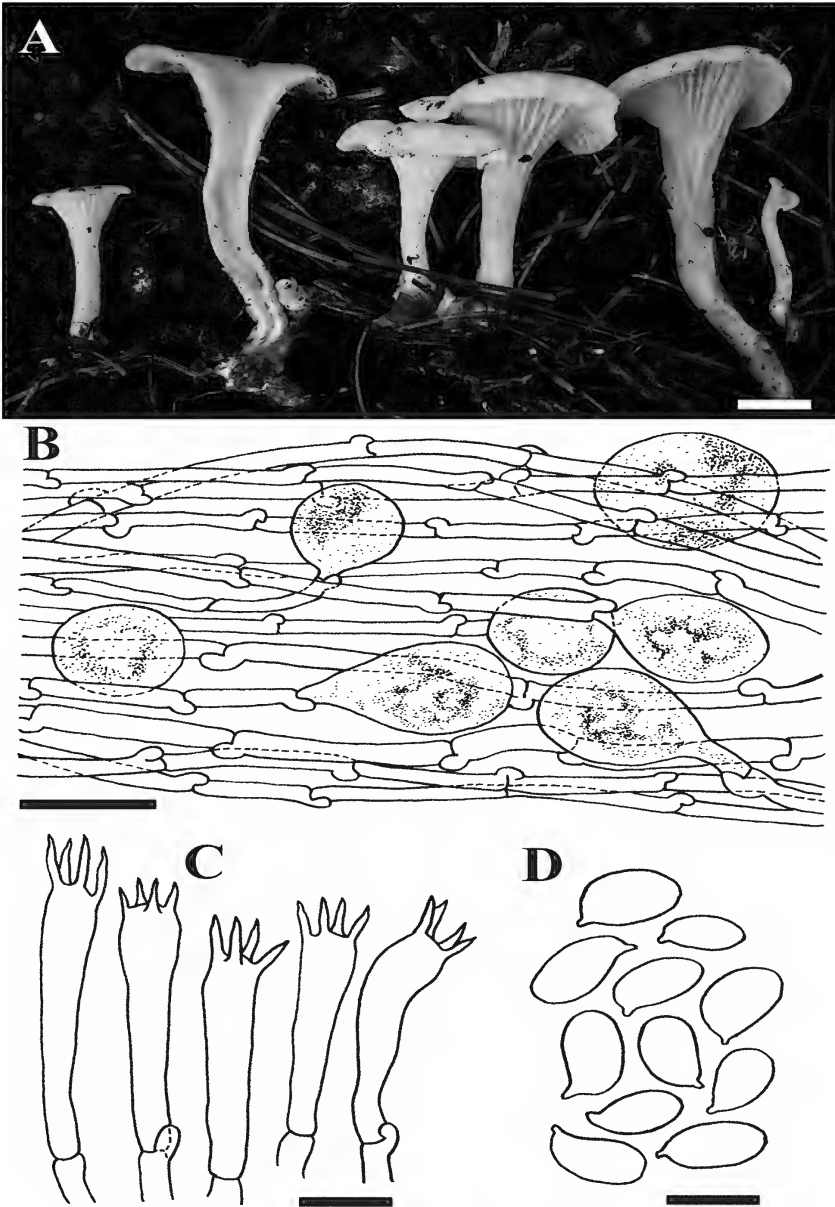


PLATE 1. *Singerocybe alboinfundibuliformis* (SDBR-CMU-SGC72). A: basidiocarps; B: pileipellis; C: basidia; D: basidiospores. Scale bars: A = 10 mm, B = 25 μ m, C = 10 μ m, D = 5 μ m.

3.0–6.5 × 2.0–4.0 µm (n = 50), Q = 1.2–2.2, elliptical, smooth, thin-walled, inamyloid. Spore print white.

SPECIMENS EXAMINED — THAILAND, CHIANG MAI PROVINCE, Muang District, Doi Suthep-Pui National Park, Yod Doi Pui, 18°49'16"N 98°53'35"E, elevation 1495 m, on rotting litter in mixed evergreen hill forest, 4 July 2015, Suwannarach N. (SDBR-CMU-SGC61; GenBank KT373850); 4 August 2015, Kumla J. & Suwannarach N. (SDBR-CMU-SGC85; GenBank KT373852); Doi Pui, 18°49'30"N 98°53'36"E, elevation 1515 m, on rich humus soil in mixed evergreen hill forest, 26 July 2015, Suwannarach N. (SDBR-CMU-SGC72; GenBank KT373851).

Molecular analysis

The ITS sequences of specimens SDBR-CMUSGC61 (702 bp), SDBR-CMUSGC72 (694 bp), and SDBR-CMUSGC85 (696 bp) were deposited in GenBank. All ITS sequences obtained in this study showed 99% similarity with *Singerocybe alboinfundibuliformis* JX514126 and JX514132.

Discussion

The presence of vesicles in the cuticle of the pileus and stipe support our collections in *Singerocybe*. All three specimens collected in northern Thailand were initially identified as *S. alboinfundibuliformis* based on descriptions by Takahashi (2000) and Seok et al. (2009) and the key provided by Qin et al. (2014). This species is easily distinguished from other *Singerocybe* species by a pileus that is deeply infundibuliform down to the base of the tubular stipe, the narrow and intervenose gills, and elliptical smooth basidiospores. *Singerocybe alboinfundibuliformis* resembles *S. humilis* (Berk. & Broome) Zhu L. Yang & J. Qin, and both are clearly distinguished from other species by their deeply infundibuliform pilei, open tubular stipes, and obviously intervenose gills (Takahashi 2000, Seok et al. 2009, Qin et al. 2014). However, *S. humilis* is separated from *S. alboinfundibuliformis* by its very narrow gills with interstices becoming transversely reticulate to subporoid and DNA sequence analyses (Qin et al. 2014). *Singerocybe humilis* occurs in South Asia, while *S. alboinfundibuliformis* appears restricted to East Asia (Takahashi 2000, Seok et al. 2009, Qin et al. 2014). *Singerocybe hydrogramma* (Bull.) Harmaja, *S. phaeophthalma* (Pers.) Harmaja, and *S. viscida* are known only from Europe (Kuyper 1981, Harmaja 1988), *S. adirondackensis* (Peck) Zhu L. Yang & J. Qin from North America (Peck 1873; Qin 2014); *S. clitocyboides* (Cooke & Massee) Zhu L. Yang et al. from Australia and New Zealand (Pegler 1965), and *S. umbilicata* Zhu L. Yang & J. Qin from East Asia (Qin et al. 2014).

The molecular analysis also confirmed the three Thai specimens as *S. alboinfundibuliformis*. Therefore, the combination of morphological and molecular characters help establish *S. alboinfundibuliformis* as a new record in Thailand.

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***Acremonium camptosporum* isolated as an endophyte of *Bursera simaruba* from Yucatan Peninsula, Mexico**

MARÍA C. GONZÁLEZ^{1*}, ANTHONY E. GLENN², RICHARD T. HANLIN³,
MARTHA L. MACÍAS RUBALCAVA⁴, BLANCA E. HERNÁNDEZ BAUTISTA⁵ &
ANA LUISA ANAYA⁵

¹ Depto. Botánica, Instituto de Biología, Universidad Nacional Autónoma de México,
Ciudad de México DF 04510 México

² USDA, ARS, Toxicology & Mycotoxin Research Unit,
Russell Research Center, Athens GA 30604 USA

³ Museum of Natural History Annex, University of Georgia,
4435 Atlanta Highway, Athens GA 30606 USA

⁴ Depto. Productos Naturales, Instituto de Química, Universidad Nacional Autónoma de México,
Ciudad de México DF 04510 México

⁵ Depto. Ecología Funcional, Instituto de Ecología, Universidad Nacional Autónoma de México,
Ciudad de México 04510, México

* CORRESPONDENCE TO: mcgv@ib.unam.mx

ABSTRACT —An interesting endophytic fungus was isolated from the leaves of *Bursera simaruba*, a tree in the semi-deciduous dry tropical forest at El Eden Ecological Reserve. The cultured strain developed the morphology characteristic of the genus *Acremonium*—tapered phialides and small aseptate conidia clustered in slimy heads. A detailed morphological examination and 28S rDNA sequence analysis identified the Yucatan strain as *Acremonium camptosporum*, an uncommon fungus found previously as a saprobe. The material is described and illustrated as the first record of this species from Mexico.

KEY WORDS — anamorphic fungi, *Ascomycota*, clavicipitaceous fungi, *Hypocreales*, taxonomy

Introduction

Acremonium is a complex and diverse genus of *Ascomycota* with widespread global distribution despite its simple morphology (Gams 1971, Glenn et al. 1996, Summerbell et al. 2011, Giraldo et al. 2014). Although *Acremonium*

species are usually isolated as saprobes from plant remains and soil, some taxa are important opportunistic human and plant pathogens (Domsch et al. 2007, Perdomo et al. 2011, Li et al. 2014, Oh et al. 2014). The systematic search for and development of new sources of chemical compounds from *Acremonium* species is also important (Rodríguez Sáiz et al. 2004, Bloemendal et al. 2014).

Bursera simaruba, a frequent co-dominant tree of tropical semi-deciduous forests in the Yucatan Peninsula, is important ecologically because it can grow in poor, clay or sandy, saline soils. Mayan communities use this plant medicinally for its analgesic, antimycotic, and anti-inflammatory properties (Gómez Pompa et al. 2003). *Bursera simaruba* exhibits a notable phenotypic variation associated with an ecotypic differentiation of some ecophysiological characters (Becerra 1999). A search for novel allelochemical compounds from endophytic fungi associated with plant communities at El Eden Ecological Reserve has revealed a distinctive endophytic ascomycete diversity that can serve as a source of new bioactive chemical compounds (González et al. 2007, 2009; Macías Ruvalcaba et al. 2008, 2010). In a survey on the diversity of fungi from El Eden Ecological Reserve, a strain (B101) with morphological characters corresponding to *Acremonium* was isolated from the leaves of *B. simaruba*.

Materials & methods

Fungus isolation and phenotypic study

El Eden Ecological Reserve is located in the State of Quintana Roo in northeastern Yucatan Peninsula (21°13'N 87°11'W). Three *Bursera simaruba* trees from a medium semi-deciduous dry tropical forest ecosystem were randomly sampled; four branches were collected from each tree for transport to the laboratory in labelled Zip-lock® plastic bags and processed within one hour of collection. One asymptomatic entire mature leaf was removed from each branch, submerged in running sterile distilled water for 60 s, and sectioned into 5 mm² pieces with sterile scissors. The leaf segments were surface cleaned by sequential washing with sodium hypochlorite solution 0.5% (v/v) (2 min) and ethanol 70% (2 min), rinsed with sterile distilled water, and surface-dried under sterile conditions (Arnold et al. 2001). Four 5 mm² leaf segments per Petri dish were plated on malt extract agar culture medium (MEA, Difco™, Becton, Dickinson & Company, Sparks, Maryland, USA) with 4g/L streptomycin sulfate and 5mg/L cyclosporine A added after autoclaving (Dreyfuss 1986). Five Petri dishes were prepared for each leaf and incubated at 25 °C under laboratory conditions and examined daily for a 28 d period. Each fungal colony developed during that period was transferred to MEA in order to obtain axenic isolates that were grouped based on their macro- and micro-morphological characters. Fourteen endophytic fungi, including isolate B101, were recovered from *B. simaruba* leaves. Isolate B101 was grown on cornmeal agar, malt extract agar, potato dextrose agar (CMA, MEA, PDA; Difco™, Becton, Dickinson and Company, Sparks, Maryland, USA), and V8 vegetable juice agar (V8A; V8 juice Campbell®, 160 mL; calcium carbonate Sigma-Aldrich®, 2 g; agar, Difco™, 20 g; distilled water to final volume of 1,000 mL)

(Hanlin & Ulloa 1988). Four Petri dishes were prepared for each culture medium and incubated at 25 ± 1 °C for 28 d. Colony macroscopic attributes (e.g., color, odor, texture, growth rate) for each tested medium were examined at low magnification with a Nikon SMZ745T stereoscopic microscope (Nikon Instruments, Tokyo, Japan) after 7, 14, 21 and 28 d. Color designations follow the Methuen handbook (Kornerup & Wanscher 1978). Microscopic morphological characters (somatic and reproductive features, hyphae, phialides, and conidia) of fungus B101 were studied by slide cultures on PDA or mounted directly in water. Photographs were made using bright field contrast (BC), dark field contrast (DC), and Nomarski differential interference contrast (DIC) with a Nikon DS digital camera using NIS software on a Nikon Eclipse 80i microscope. Images were processed with Adobe Photoshop CS6®. Also, the fungus was studied with a Jeol 5410LV scanning electron microscope according to Goh & Hanlin (1994).

DNA isolation, PCR amplification, sequencing, and phylogenetic analysis

Genomic DNA was extracted for molecular analyses from the endophyte fungus B101 (Glenn et al. 1996). The nuclear 28S large subunit RNA gene (LSU) was amplified with primers LR0R and LR6 and sequenced with primers LROR, LR3, LR3R, LR5, and LR6 (Vilgalys & Hester 1990, Rehner & Samuels 1995). The DNA was sequenced by the USDA-Agricultural Research Service, South Atlantic Area Sequencing Facility (Athens, GA, USA). The LSU sequence from B101 was compared with GenBank sequences using the Geneious v7.1.8 Linnaeus Blast search feature. The compiled LSU dataset was aligned using MUSCLE, also all within Geneious. The alignment was evaluated using MEGA v.6.06 to determine the maximum likelihood nucleotide substitution model having the best fit. Model TN93 (including estimated transition/transversion ratio, proportion of invariable sites, and gamma distribution parameter) was used for PhyML analysis to identify the maximum likelihood cladogram, which was assessed by 200 bootstrap replications. Model GTR with the same estimated parameters was used for MrBayes analysis (10,000,000 chain length; 4 heated chains; 0.2 heated chain temp; 200 subsampling frequency; 0.125 burn-in length). PhyML and MrBayes assessments were conducted within Geneious. Sequences from five species of *Cosmospora/Nectria* were designated as the outgroup.

Fungal specimen

The Mexican endophytic fungus (B101) was deposited in the Colección de Hongos of the Herbario Nacional de México, Universidad Nacional Autónoma de México, Distrito Federal, México (MEXU). In addition, a specimen from the tree from which B101 was isolated was deposited in the Herbarium, University of California, Riverside CA, U.S.A. (G.P. Schultz & R. Palestina # 1092/UCR 110695). The 28S rDNA sequence generated in this study was deposited in GenBank.

Taxonomy

Acremonium camptosporum W. Gams, Cephal.-art. Schimmelp.: 57. 1971. Figs 1–4

MYCELIAL COLONIES in vitro growing 30–35 mm diam after 28 d on PDA at 25 °C, flat to slightly elevated in center, funiculose to floccose textured, white (1A1) to yellowish white (1A2), reverse colored with pale yellow ochraceous to

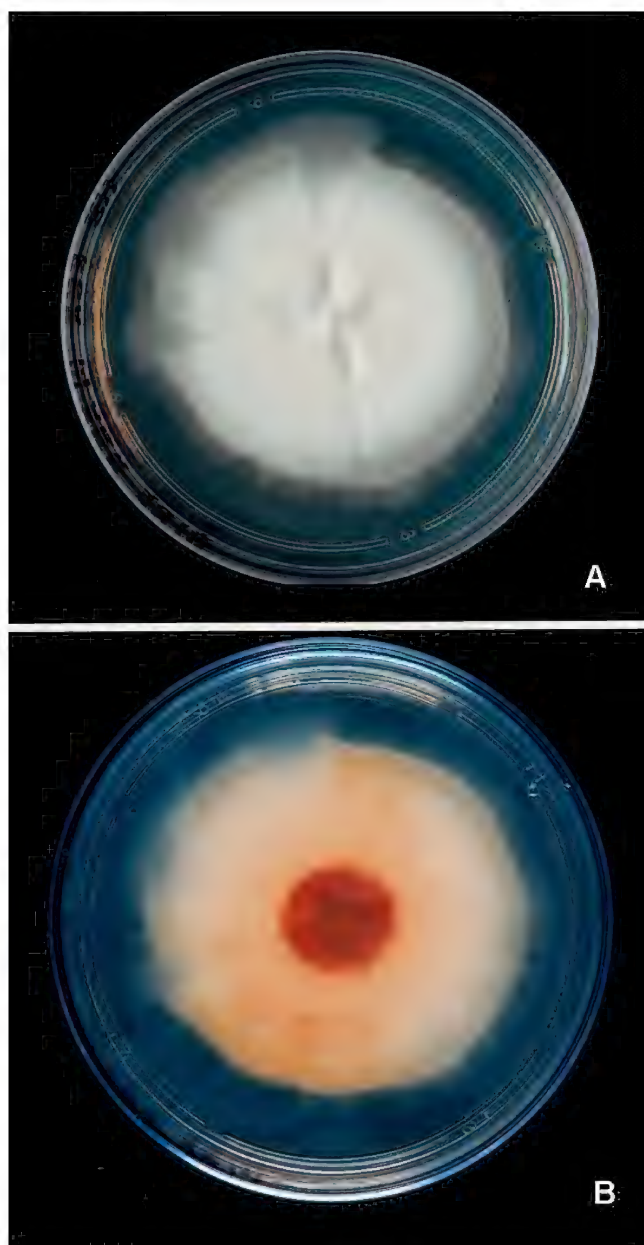


FIG. 1. *Acremonium camptosporum* (MEXU 26354). A. obverse of colony on potato dextrose agar after 28 d at 25 °C; B. reverse of same culture with ochre-brown pigment.

dark yellow ochraceous central area. Cubical or elongated crystals numerous. Odor not pronounced. SOMATIC HYPHAE septate, hyaline, smooth- and thin-walled, slightly to highly convoluted, often anastomosed, 0.5–2.5 µm wide, plectonematogenous. CONIDIOPHORES often basitonously branched, sporodochia not observed, sporulation abundant. PHIALIDES straight or slightly flexuous, 15–46 µm long, 0.5–1 µm wide at the base, tapering to 0.4–0.5 µm at tip, without a chromophile base, collarete or periclinal thickening at the apex, hyaline, thin- and smooth-walled, slightly constricted at basal septum, orthophialides formed, prostrate phialides present, adelophialides and schizophialides not observed. CONIDIA aseptate, hyaline, guttulate, smooth- and thin-walled, heteropolar-bilateral, mostly plano-convex or asymmetrically biconvex in front view, 1.5–2.5(–2.8) µm long, 0.8–1.1 µm wide, fusiform in side view, sometimes slightly curved and basally apiculate, aggregated in small slimy globose clusters, catenate conidia not seen. CHLAMYDOSPORES and SEXUAL MORPH not observed.

MATERIAL EXAMINED: MEXICO. QUINTANA ROO: Isla Mujeres Municipality, El Eden Ecological Reserve, 21°13'N 87°11'W, from leaves of *Bursera simaruba* (L.) Sarg. (*Burseraceae*), May 2004, MC González, AL Anaya (MEXU 26354; GenBank JQ811556).

Phylogenetic analysis & discussion

Maximum likelihood (ML) and Bayesian analyses support MEXU 26354 and the *A. camptosporum* isolates within the *Clavicipitaceae* s.l., with particular association with *Elaphocordyceps* and *Metacordyceps* (FIG. 6). Bayesian support was strong for this large clade, but there was no substantial support of this relationship from the ML analysis. In general the Bayesian analysis was more supportive of the major clades than was ML. The relationship between MEXU 26354 and the other *A. camptosporum* isolates was very well supported (Bayesian posterior probability = 1; ML bootstrap = 100%). The LSU sequence of MEXU 26354 was 99.5–99.7% identical to the four *A. camptosporum* accessions.

According to Summerbell et al. (2011), *Acremonium* can be morphologically characterized by its conidiophores that are mainly unbranched or poorly basitonously branched and subglobose, obovate– ellipsoidal conidia, with adelophialides usually absent. However, they conclude that because of the relatively common plasticity of form in *Acremonium* and acremonioid species, the distinctive LSU sequences for delineating the species phylogenetically, precise morphological observation, and correct interpretation of the morphological descriptions are all necessary.

Gams (1971) described the type specimen of *Acremonium camptosporum* from Kiel-Kitzeberg, Germany; his description and drawings depict structures that are similar with those produced by the Mexican collection MEXU 26354.

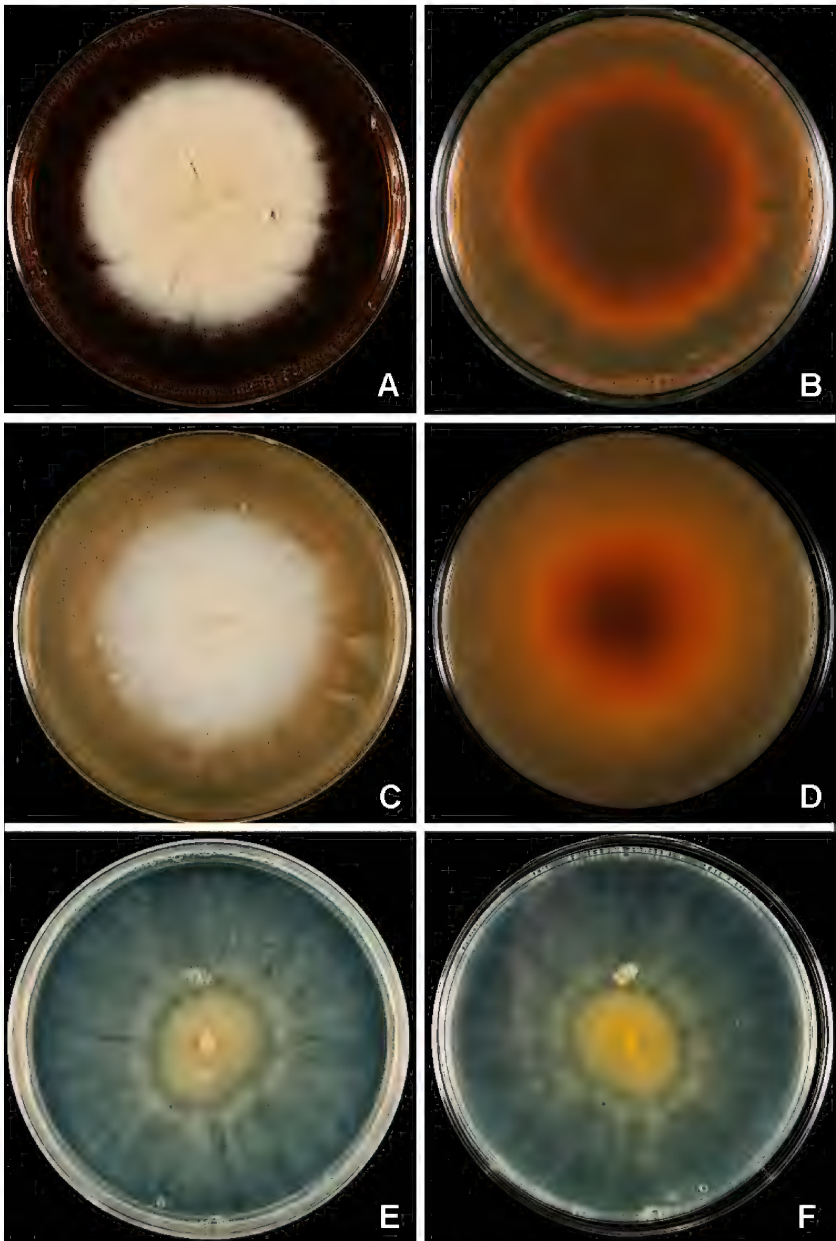


FIG. 2. *Acremonium camptosporum* (MEXU 26354). A, C, E: obverse of colonies on malt extract agar, V-8 juice agar, and corn meal agar after 28 d at 25 °C; B, D, F reverse of same cultures.

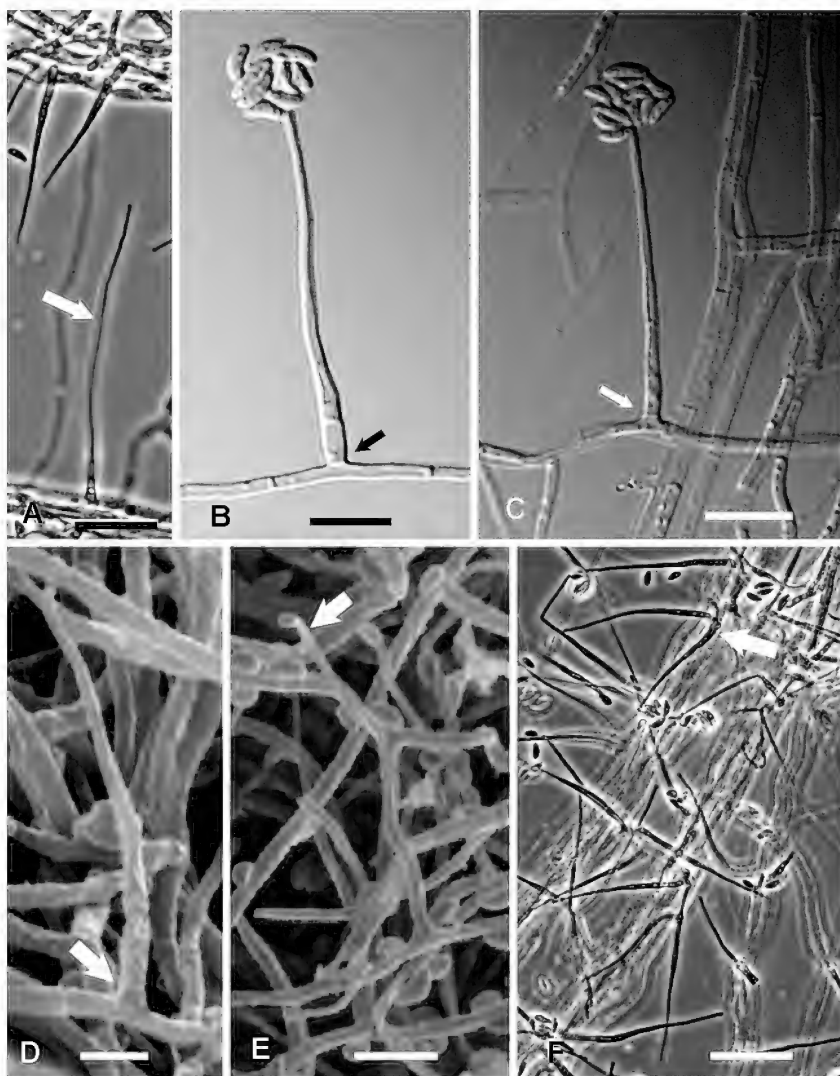


FIG. 3. *Acremonium camptosporum* (MEXU 26354). A. phialides – arrow indicates one longer and narrower; B. phialide slightly constricted at basal septum (arrow) and conidia clustered in slimy head; C. D. orthophialide with basal septum (arrow); E. flexuous phialide with phialospore attached at tip (arrow); F. pleptonematogenous sporulation, phialides unbranched and branched (arrow). Scale bars: A = 10 µm; B–E = 5 µm; F = 20 µm.

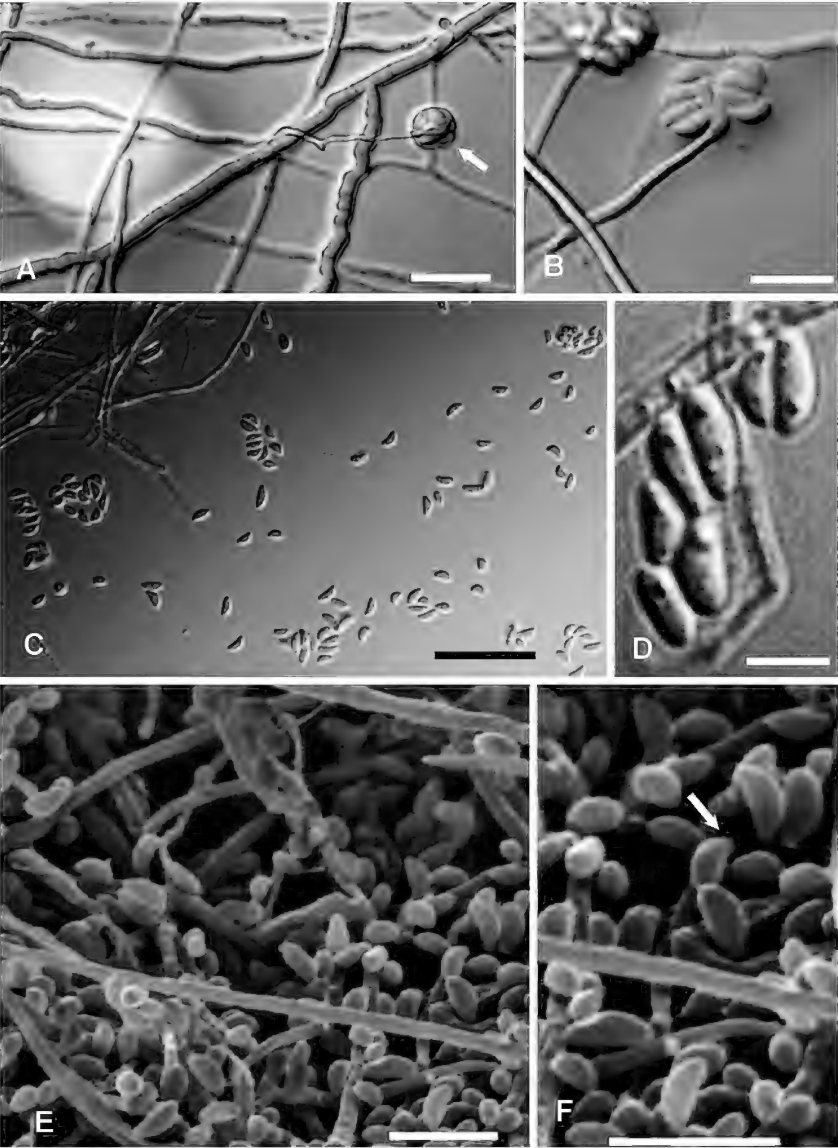


FIG. 4. *Acremonium camptosporum* (MEXU 26354). A. tiny prostrate hypha with subterminal phialide and conidia clustered in subglobose slimy head (arrow); B. phialidic development of curved phialoconidia; C. small hyaline curved conidia; D. guttulate conidia; E. aseptate conidia; F. slightly curved and basically apiculate (arrow) and plano-convex conidiospores. Scale bars: A, C = 10 µm; B, E, F = 5 µm; D = 2 µm.

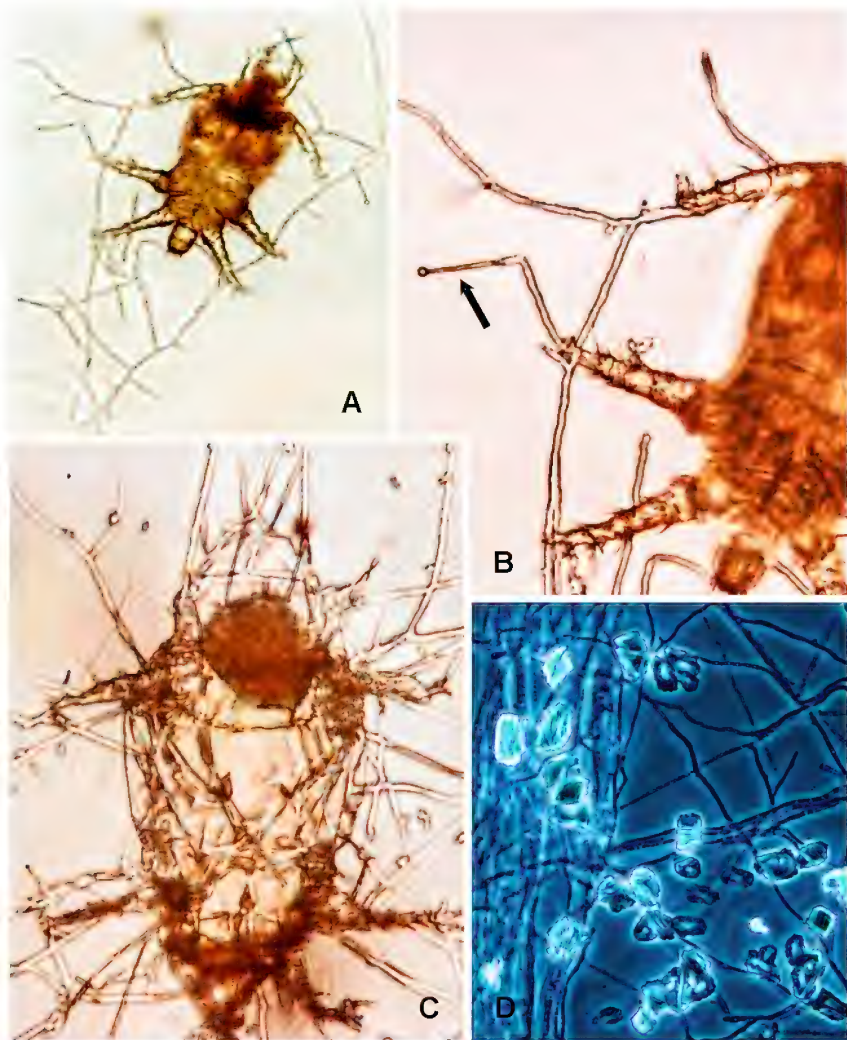


FIG. 5. *Acremonium camptosporum*. A. dead mite attached to internal lateral wall of Petri dish by *A. camptosporum* hyphae from edge of 6 wk old pure culture; B. detail of mite legs with hyphae of *A. camptosporum*; note a phialide with globose mass of conidia (arrow); C. dead mite in advanced state of degradation by *A. camptosporum*; D. cubical and irregular crystals.

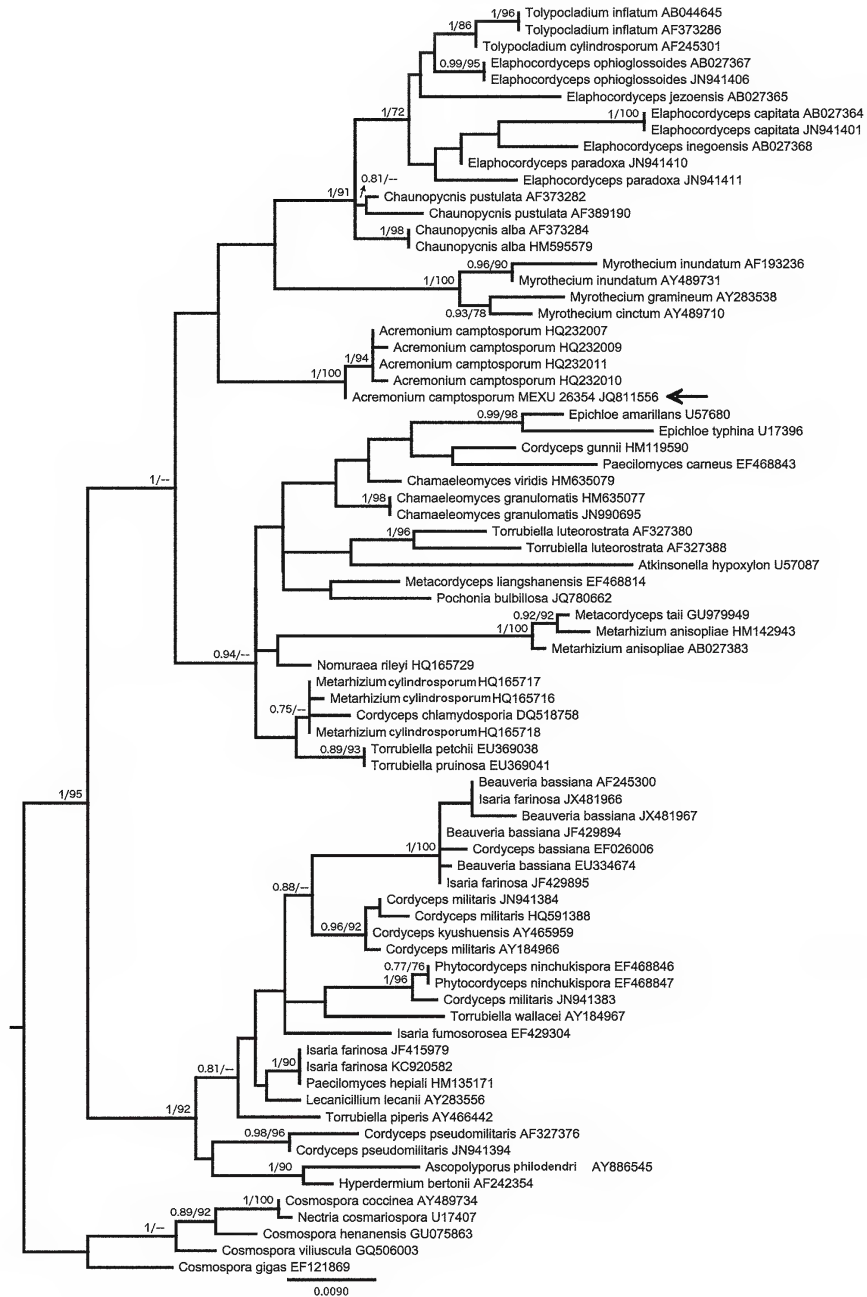
As described by Gams (1971), *A. camptosporum* is characterized by slow-growing white colonies with a yellow ochre reverse, the presence of orthotropic phialides, and small curved conidia arranged in slimy heads. The Mexican specimen has the same micro-morphological characteristics and agrees with

the *A. camptosporum* description, particularly with the variable form of its conidia (slightly curved and basally apiculate, heteropolar-bilateral, plano-convex or asymmetrically biconvex) and the presence of characteristic crystals (FIG. 5D). However, the Mexican material has conidia that are minutely guttulate (sometimes with one or two conspicuous guttules) and slightly smaller, with maximum measurement values corresponding to the minimum values mentioned in Gams's description (2.8–3.8 μm long $\times$ 1.1–1.8 μm wide). Gams (1971) also noted the presence of sporodochial conidiomata in older cultures—not observed in our cultures, although delimited higher sporulation zones were noted in central area of colonies. Although the conidia of MEXU 26354 are smaller than those of the type description, based on our phenotypic and phylogenetic analyses, we conclude that our material represents *A. camptosporum*.

Gams (1971) mentioned that *A. camptosporum* shares some morphological characters with *Xenoacremonium recifei* [$\equiv$ *Acronium recifei* = *Cephalosporium recifei* = *Hyalopus furcatus* = *Hyalopus recifei*], a tropical opportunistic pathogen of humans characterized by aseptate, hyaline, ellipsoidal to fusiform or reniform, slightly curved conidia, but *X. recifei* can be distinguished by its shorter conidia without periclinal thickening, slower colony development, and intensive pigmentation of the colony reverse.

Our results support a close phylogenetic relationship between *Acronium camptosporum* and the clade encompassing *Ophiocordycipitaceae* and *Clavicipitaceae* s. str. (Sung et al. 2007) (FIG. 6). Interestingly, our study also nests the *A. camptosporum* clade with the *Elaphocordyceps* clade. Data analysis suggests that *A. camptosporum* is basal to *Elaphocordyceps* and other fungi with asexual morphology in the *Ophiocordycipitaceae*. The *Elaphocordyceps* clade contains the asexual genera *Tolypocladium* and *Chaunopycnis*. *Tolypocladium* species typically produce smooth globose or cylindrical conidia on inflated-base phialides with slightly tapering apex. They are parasites of the ectomycorrhizal truffle-like genus *Elaphomyces*, entomopathogens of cicada nymphs buried in soil, and scarab beetle larvae embedded in decaying wood and are also isolated from soil (Gams 1971, Sung et al. 2007). *Chaunopycnis* species produce minutely roughened globose or obovate conidia on cylindrical or inflated-base phialides slightly tapering in the distal part, formed in conidiomata isolated

FIG. 6. LSU sequence cladogram (PhyML) showing the relationship of *Acronium camptosporum* to other taxa within the *Clavicipitaceae* sensu lato. Numbers above branches indicate the Bayesian posterior probability support (>0.75) followed by the bootstrap support (>75%) from 200 maximum likelihood replicates.



from soil, plant litter, crustose lichen thalli of *Caloplaca regalis*, and living leaves of *Kalmia latifolia* (Gams 1980, Möller & Gams 1993, Bills et al. 2002). The Mexican *A. camptosporum* strain (MEXU 26354) differs morphologically and ecologically from those taxa. More robust statistical analyses still needed for a higher-level phylogeny of *A. camptosporum*.

The endophyte strain of *A. camptosporum* (MEXU 26354) produces six secondary metabolites of three different heterodimeric polyketide groups, one of which is acremoxanthone E, a new compound that shows biological activity against selected phytopathogens and human tumor cell lines (Meléndez-González et al. 2015).

Acremonium camptosporum is a rarely isolated fungus, previously reported only from air in Germany (Gams 1971); soil in Czech Republic (Řepová 1989), Ivory Coast (Persiani et al. 1998), and Brazil (Prade 2007); and anthropogenic substrates in Czech Republic (Váňová & Bečvář 1998) and Poland (Blyskal 2009, 2015). Additional nucLSU sequences from leaf litter of *Acacia karroo* soil in South Africa (CBS 757.69, CBS 677.74), from insects in Cuba (CBS 835.91), and from soil nematodes in Germany (CBS 890.85) give a broad view of *A. camptosporum* distribution and ecology. This is both the first record of *A. camptosporum* from Mexico and the first record of the species as a leaf endophyte.

Melanins function in protecting fungi from adverse environmental factors (Gómez & Nosanchuk 2003). Some *Sarocladium* species have an ochre-brown or dark grey-brown colony reverse due to melanin synthesis (Summerbell et al. 2011). The Mexican *A. camptosporum* strain produces yellow ochre to ochre-brown pigments on the reverse of colonies cultured. The highest color intensity was expressed on MEA, whereas it was colorless on CMA (Fig. 2B, F).

In a recent study on biodeterioration of cochineal dyed antique woolen textiles, *A. camptosporum* caused significant degradation of wool fibers due to its ability to utilize keratinous materials (Blyskal 2009, 2015). The keratin-associated proteins and high glycine-tyrosine proteins of wool fibers are a good nutritional resource for small arthropods (e.g., mites), and keratins for fungi and other microorganisms. Interestingly, we observed here the apparent degradation of a dead mite by the *A. camptosporum* MEXU 26354 endophytic strain (Fig. 5A–C).

The smaller somatic and reproductive structures of the *A. camptosporum* endophytic strain from *Bursera simaruba* leaves of Yucatan Peninsula, along with its apparent keratinophilic ability, suggest a possible adaptation to its niche. More studies are necessary to clarify the ecology of this fungus.

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***Aspicilia*, *Lobothallia*, and *Rhizocarpon* species new for Turkey and Asia**

KENAN YAZICI ^{*1} & ALI ASLAN ²

¹Biology Department, Faculty of Science, Karadeniz Technical University,
61080, Trabzon, Turkey

²Atatürk University, Kazım Karabekir Education Faculty, Biology Dept., Erzurum, Turkey

* CORRESPONDENCE TO: kcagri_1997@yahoo.com

ABSTRACT — Three lichen species, *Aspicilia glomerulans*, *Lobothallia chadefaudiana*, and *Rhizocarpon furfurosum*, were identified as new to Turkey and Asia during a lichenological survey in the Burdur and Muş regions (Turkey). Descriptions accompany notes on known distribution, substrates, and chemistry and comparisons with morphologically similar taxa.

KEY WORDS — ascomycetes, biodiversity, *Hymeneliaceae*, *Megasporaceae*, *Rhizocarpaceae*

Introduction

Although many new lichen species have recently been recorded for Turkey (Aptroot & Yazici 2012, Arslan et al. 2011, Yazici et al. 2010a, b, c, 2011a, b, 2012, Karagöz & Aslan 2012, Karagöz et al. 2011, Kinalioğlu & Aptroot 2011, Osyczka et al. 2011, Vondrák et al. 2012), more surveys are still needed of unexplored regions in the country.

Rhizocarpon (*Rhizocarpaceae*) contains more than 200 crustose species (Miadlikowska et al. 2014, McCarthy & Elix 2014), which are found on siliceous rocks in montane and boreal habitats and (rarely) on substrates in wet or dry (sub)tropical areas and hot-arid regions (McCarthy & Elix 2014). *Aspicilia* (*Hymeneliaceae*) contains approximately 230 species (Nordin et al. 2010), while *Lobothallia* (*Megasporaceae*) has 12 lichen taxa (Sharnoff 2014).

Thirty-eight taxa of *Aspicilia*, three taxa of *Lobothallia*, and 30 taxa of *Rhizocarpon* have thus far been reported from Turkey. Of the approximately 1650 lichen taxa recorded for the country, only 37 lichenized fungi have been

reported from Burdur Province (Aslan & Yazıcı 2013, Çobanoğlu 2005, Öztürk et al. 2005, Pišút & Guttová 2008, Şenkardeşler 2009, Yazıcı et al. 2013).

The present contribution results from fieldwork in the regions of Burdur, southwestern Turkey, and Muş, eastern Turkey. We report here three new records for Turkey and Asia.

Materials & methods

Specimens

Air-dried samples were examined with a Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i compound light microscope. Relevant keys were consulted (McCarthy & Elix 2014, Clauzade & Roux 1985, Poelt 1970, Roux 1977, Smith et. al. 2009) for the identifications. Vouchers are stored in the Herbarium of the Biology Department, Karadeniz Technical University, Trabzon, Turkey (KTUB). The descriptions are based on Turkish specimens.

Description of the study area

Areas visited were the Ağlasun, Altınyayla, and Tefenni districts in Burdur and the center and Bulanık and Varto districts in Muş. Burdur, which is very mountainous and covered with mixed forests (*Abies*, *Cedrus*, *Ficus*, *Fraxinus*, *Juniperus*, *Olea*, *Pinus*, *Pistacia*, *Prunus*), has a continental Mediterranean climate with cold snowy winters and long dry and very hot summers. Muş province, which encompasses vast areas of grassy meadows and steppe and high mountains with occasional to rare *Quercus* communities and *Salix* trees, has a climate characterized by very cold snowy winters, and short hot dry summers (Baytop & Denizci 1963, Akman 1999).

Taxonomy

Aspicilia glomerulans (Poelt) Poelt, Mitt. Bot. Staatssamml. München
8: 202 (1970)

FIG. 1

Thallus crustose, to 7 cm diam, K–, areolate, with black hypothallus, rounded, 0.5 mm thick, dark white or dark-grey, paler at the middle, dark grey-brown towards the outer margin of the lobes; surface with numerous isidia-like papillose branches, dispersed or aggregated on one areole or many areolae; apothecia present or absent, 0.8–1 mm diam., single, rounded, disc flat, concave later, black, edge flat, black, ± rounded, disappearing later; ascospores ellipsoid, 16–19 × 6–12 µm, with granules; hymenium 130 µm high, pale, I+ blue; asci cylindrical, 8-spored, thick and contrasting tholus, strongly regressed at maturity, I ± blue; paraphyses barely in water, upper parts moniliform and anastomosing in K.

ECOLOGY—Associated species: *Aspicilia cinerea*, *Candelariella vitellina*, *Melanohalea infumata*, *Parmelina tiliacea*, *Physcia caesia*, *P. cernohorskyi*, *Rhizoplaca chrysoleuca*, *R. melanophthalma*, and *Xanthoria elegans*.

SPECIMEN EXAMINED – TURKEY. BURDUR: ALTINYAYLA, between İbecik-Altınyayla mainroad side, 36°58'07.19"N 29°26'17.70"E, 1348 m, on calcareous rock, 29.06.2012.

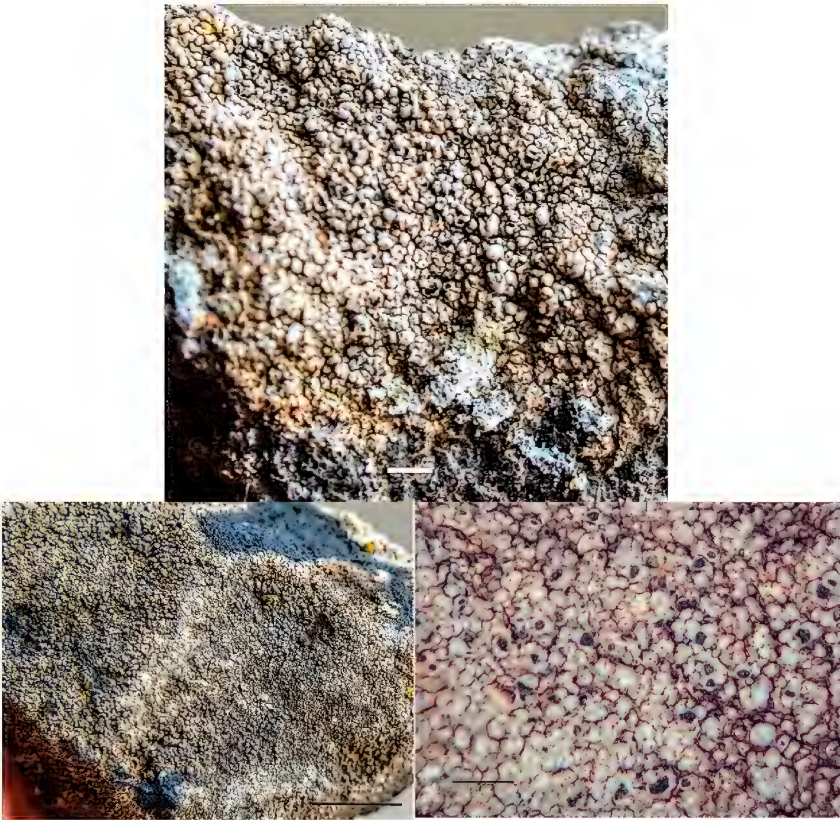


FIG. 1. *Aspicilia glomerulans*, habitus
(top: with apothecia & papilla; left: with papilla; right: with apothecia & papilla).
Scales top, right = 1 mm; left = 1 cm

leg. K.Yazici. (KTUB-2427), Muş: VARO, Çallidere village, roadside, 39°16'24.70"N
41°14'18.26"E, 1705 m, on calcareous rock, 01.07.2015, leg. K.Yazici. (KTUB-2445).

REMARKS—*Aspicilia glomerulans*, which is primarily sterile, grows on calcareous rock. The species is morphologically similar to *Koerberiella wimmeriana*, which differs by its grey-rose to grey thallus, cylindrical papillose isidia, shorter pycnidiospores ($4-5 \times 1 \mu\text{m}$), larger apothecia (0.5–2 mm in diam.), and larger ascospores ($20-35 \times 11-20 \mu\text{m}$) (Clauzade & Roux 1985, Poelt 1970, Thüs & Schultz 2009). For additional details, see Poelt (1970).

Aspicilia glomerulans has previously been reported from Austria (Alps), Greenland, North America, and Norway (Clauzade & Roux 1985, Poelt 1970). This is a first record for Turkey and Asia.



FIG. 2. *Lobothallia chadefaudiana*, habitus (apothecia and thallus lobes). Scale = 1 mm

Lobothallia chadefaudiana (Cl. Roux) A. Nordin, Cl. Roux & Sohrabi, Bull. Soc.

Linn. Provence, num. spéc. 16: 216 (2012)

FIG 2

Thallus crustose, pale white to grey white, 1.7– 3 cm in diam., squamules, to 2 mm in diam.; surface with papillose, $\pm$ pruinose, rounded, $\pm$ lobed, spot tests negative; cortex ≤ 85 μm high, photobiont *Trebouxia*. Medulla ≤ 1300 μm ; apothecia to 2 mm diam., $\pm$ immersed, in squamules in 1–2 groups, irregular in shape, disc concave, flat later, $\pm$ grey-blue, pruinose, K–, P–, C–; N–, with paraphyses with gelatinous sheath at the top, K–; hymenium 80–95 μm , colourless, without crystals, paraphyses 3–6 μm thick, strongly coherent, consistent, divided, green-brown, branched and anastomosing, I + blue later red; hypothecium 35–55 μm , colourless; asci clavate, thickened at the top, 80–95 $\times$ 20–30 μm , 8-spored, I+ blue, later red. Ascospores 9–17 $\times$ 6–12 μm .

ECOLOGY—Associated species: *Acarospora cervina*, *Lecanora dispersa*, *Verrucaria nigrescens*, and *Diploschistes diacapsis*.

SPECIMEN EXAMINED – TURKEY. BURDUR: AĞLASUN, 5 km to Çamlidere village, 37°38'01.77"N 30°38'53.58"E, 1010 m, on calcareous rock, 17.07.2012, leg. K.Yazıcı. (KTUB–2423), MUŞ: CENTER, 200 m to Alaniçi village, roadside, 38°39'15.52"N 41°21'10.55"E, 1709 m, on calcareous rock, 29.05.2015, leg. K.Yazıcı. (KTUB–2447).

REMARKS— *Lobothallia chadefaudiana* mostly grows on calcareous rock. It is morphologically similar to *Aspicilia cernohorskyana*, which is distinguished by larger apothecia and narrower squamules (Clauzade & Roux 1985, Roux 1977). For additional details, see Roux (1977).

Lobothallia chadefaudiana has previously been reported from Europe (France, Austria, Greece, Spain; Clauzade & Roux 1985, Roux 1977, Roux et al. 2008). This is a first record for Turkey and Asia.



FIG. 3. *Rhizocarpon furfurosum*, habitus (with isidia). Scale = 1 mm

Rhizocarpon furfurosum H. Magn. & Poelt, Verh. Zool.-Bot. Ges. Wien
95 : 110 (1955)

FIG. 3

Thallus crustose, effuse, to 1.2–4 cm diam., continuous to rimose-areolate, areoles to 1.2 mm diam., cracked, spreading, pale or dull-brown-grey to dark grey-brown, prothallus ± developed, covered with fragile isidia like blastidia minutely warted at first; isidia 0.01–0.03 mm long, fragile, branched. Areoles flat or ± convex. Hypothallus black. Apothecia absent. Thallus K+ yellow, C–; medulla K+ yellow, P+ yellow.

ECOLOGY—Associated species: *Acarospora cervina*, *Bellemeria cupreoatra*, *Candelariella vitellina*, *Lecanora dispersa*, *Protoparmeliopsis muralis*, *Rhizocarpon geographicum*, and *Xanthoria elegans*.

SPECIMEN EXAMINED – TURKEY. BURDUR: TEFENNI, Seydiler village, 37°14'47.46"N 29°48'24.83"E, 1191 m, on siliceous rock, 21.08.2013, leg. K.Yazıcı. (KTUB–2429), MUŞ: BULANIK, 5 km to Arakonak village, roadside 39°04'08.79"N 42°07'48.39"E, 1602 m, on calcareous rock, 30.06.2015, leg. K.Yazıcı. (KTUB–2446).

REMARKS— *Rhizocarpon furfurosum*, which occurs on metal-rich siliceous rocks, is mostly sterile and is easy to overlook. For additional details, see Smith et al. (2009).

Rhizocarpon furfurosum has previously been reported from Europe (England, Germany, Scandinavia, the Czech Republic, Slovakia, Russia; Guttová et al. 2013, Smith et al. 2009 Steinová et al. 2009). This is a first record for Turkey and Asia.

Acknowledgements

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New species of neotropical *Rhodocollybia*

JUAN L. MATA^{1*}, CLARK L. OVREBO²,
TIMOTHY J. BARONI³ & KAREN W. HUGHES⁴

¹Biology Department, University of South Alabama,
5871 USA Dr. N, Room 124, Mobile, AL, 36688

²Department of Biology, University of Central Oklahoma,
100 North University Drive, Edmond, OK 73034

³Department of Biological Sciences, State University of New York – College Cortland,
P.O. Box 2000, Cortland, NY 13045

⁴Department of Ecology and Evolutionary Biology, University of Tennessee,
569 Dabney Hall, Knoxville, TN 37996

* CORRESPONDENCE TO: jmata@southalabama.edu

ABSTRACT —. Sequences from the internal transcribed spacer region of the ribosomes helped circumscribe two new *Rhodocollybia* species for neotropical America: *R. olivaceogrisea* from Costa Rica; and *R. tenuipes* from Dominican Republic and Puerto Rico. Both species are phylogenetically related to *R. butyracea*. *Rhodocollybia maculata* represents a new report for Dominican Republic.

KEY WORDS — ITS, phylogeny, *Omphalotaceae*, *Agaricomycetes*

Introduction

The genus *Rhodocollybia* Singer (*Omphalotaceae*, *Agaricomycetes*) comprises fleshy mushrooms with convex smooth pilei with crowded cream-colored lamellae, striate and slightly twisted stipes, and a pinkish spore deposit (Antonín & Noordeloos 2010). Basidiospores are at least partly, dextrinoid. However, not all *Rhodocollybia* mushrooms explicitly exhibit this entire set of characters and sometimes may be confused with closely related genera, such as *Gymnopus* (Hughes et al. 2010). In addition to detailed morphological analyses, DNA studies are very helpful determining identities of many mushrooms whose species boundaries are difficult to resolve.

Most reports for *Rhodocollybia* are from temperate regions (Antonín & Noordeloos 2010, Cooper 2014, Halling 2009, Lee et al. 2014, Lennox 1979). Studies by Halling (1989) and Mata et al. (2004) thus far provide the best accounts for neotropical America.

A recent foray in Costa Rican oak-dominated forests confirmed a suspected new species of *Rhodocollybia*. Previous studies of Caribbean material also suggested new records. Here we propose two new neotropical species: *R. olivaceogrisea* from Costa Rica and *R. tenuipes* from Dominican Republic

TABLE 1. *Rhodocollybia* and outgroup sequences used in the ITS phylogenetic analysis. New sequences are set in bold font.

TAXON	GENBANK NO.	VOUCHER	REFERENCE
<i>Lentinula raphanica</i> (Murrill) J.L. Mata & R.H. Petersen	AY016441	TFB 9156	Mata et al. 2001
<i>Gymnopus luxurians</i> (Peck) Murrill	KJ416241	TFB 14107	Petersen & Hughes 2014
<i>R. amica</i> J.L. Mata & Halling	AF505754	TFB 9920	Mata et al. 2004
<i>R. butyracea</i> (Bull.) Lennox	GU318386	TFB 13006	Hughes et al. 2010
	KJ609163	SFC20120821-75	Lee et al. 2014
	AY313290	TFB 8801	Mata et al. 2004
	AY313293	TFB 7452	Mata et al. 2004
	DQ444317	OKM27562	Mata et al. 2007
	AY313292	TFB 8250	Mata et al. 2004
<i>R. dotae</i> J.L. Mata & Halling	AF505758	REH 7007	Mata et al. 2004
<i>R. laulaha</i> Desjardin et al.	GU369944	SFSU: DED6393	Keirle et al. 2010
<i>R. lignitilis</i> J.L. Mata & Halling	AF505753	REH 7907	Mata et al. 2004
<i>R. maculata</i>	KT205402	TFB 11720	This paper
	AY313296	TFB11045	Mata et al. 2004
	AF505756	TFB 9605	Mata et al. 2004
	GU947368	BRNM 707085	Antonín & Noordeloos 2010
	GU947370	BRNM 699408	Antonín & Noordeloos 2010
	GU947369	BRNM 714632	Antonín & Noordeloos 2010
<i>R. olivaceogrisea</i>	KT205399	JLM 2175	This paper
	KT205400	CLO 4368	This paper
<i>R. pandipes</i> Halling & J.L. Mata	KT205401	JLM 2251	This paper
	AY313295	TFB 9680	Mata et al. 2004
	AF505752	TFB 11014	Mata et al. 2004
	AY313294	TFB 7899	Mata et al. 2004
<i>R. prolixa</i> var. <i>distorta</i> (Fr.) Antonín et al.	AF505748	EFM1403	Mata et al. 2004
<i>R. purpurata</i> (G. Stev.) J.A. Cooper	KJ61902	PDD:95837	Cooper 2014
<i>R. tenuipes</i>	AY313288	TFB 11707	Mata et al. 2004
<i>R. tablensis</i> J.L. Mata & Halling	AF505755	EN2066	Mata et al. 2004
<i>R. turpis</i> (Halling) Halling	AF505749	FB10077	Mata et al. 2004
<i>R. unakensis</i> (Murrill) Halling	AY313298	TFB 10482	Mata et al. 2004

and Puerto Rico. Phylogenetic analysis nests both species in a clade with *R. butyracea*. *Rhodocollybia maculata* is a new report for the Dominican Republic, and it and *R. tenuipes* are the first *Rhodocollybia* records from this country.

Materials & methods

Morphology

Basidiomata colors in capital alphanumeric codes in square brackets are from Kornerup & Wanscher (1978) and small-cap codes are from Kelly (1965). Preparation for tissues and basidiospores for microscopy observations and descriptive abbreviations are as in Mata et al. (2004). Line drawings were generated using a drawing tube attached to an Olympus CX41 microscope.

Phylogeny

DNA extraction and PCR protocols are as those used in Mata et al. (2004). Ribosomal ITS-4 and ITS-5 primers were used to amplify the internal transcribed region (ITS) of nuclear DNA (White et al. 1990). PCR products were purified with Qiaquick PCR Purification Kit (Qiagen®). Purified PCR products were sequenced at Macrogen (MD, USA). Sequence files were first edited with Geneious R8 (Geneious 2014) and then blasted against NCBI nucleotide databases. Selected *Rhodocollybia* sequences were downloaded together with one of *Lentinula raphanica* and *Gymnopus luxurians*, which were used as the outgroup (TABLE 1). The matrix was aligned with MAFFT (Katoh et al. 2002). Maximum likelihood phylogenetic tree was calculated with PhyML (Guindon & Gascuel 2003) using 1000 bootstrap replications and supported with posterior probabilities using Mr. Bayes (Huelsenbeck & Ronquist 2001). All programs were run with Geneious R8.

Abbreviations

Country abbreviations are as follow: Costa Rica (CRC), Czech Republic (CZR), Dominican Republic (DOM), Italy (ITA), Korea (KOR), Mexico (MEX), New Zealand (NZL), Sweden (SWE), and United States (USA). Herbarium acronyms where collections are deposited are according to Thiers (2015).

Results

Phylogeny

ITS sequences of *R. olivaceogrisea* nest in a clade with those of *R. butyracea* (a well-known species in temperate regions), but its closest relative appears to be *R. pandipes* from Costa Rica (PLATE 1). Percent identity between these two species is 95.39% based on the distance matrix. Sequences from both *R. olivaceogrisea* collections are 99.73% identical, with one representing a collection from the Talamanca Mountains and the other from the Central Volcanic Range of Costa Rica. The *R. tenuipes* sequence from the Dominican Republic also nests in the clade with *R. olivaceogrisea* and *R. butyracea* but is

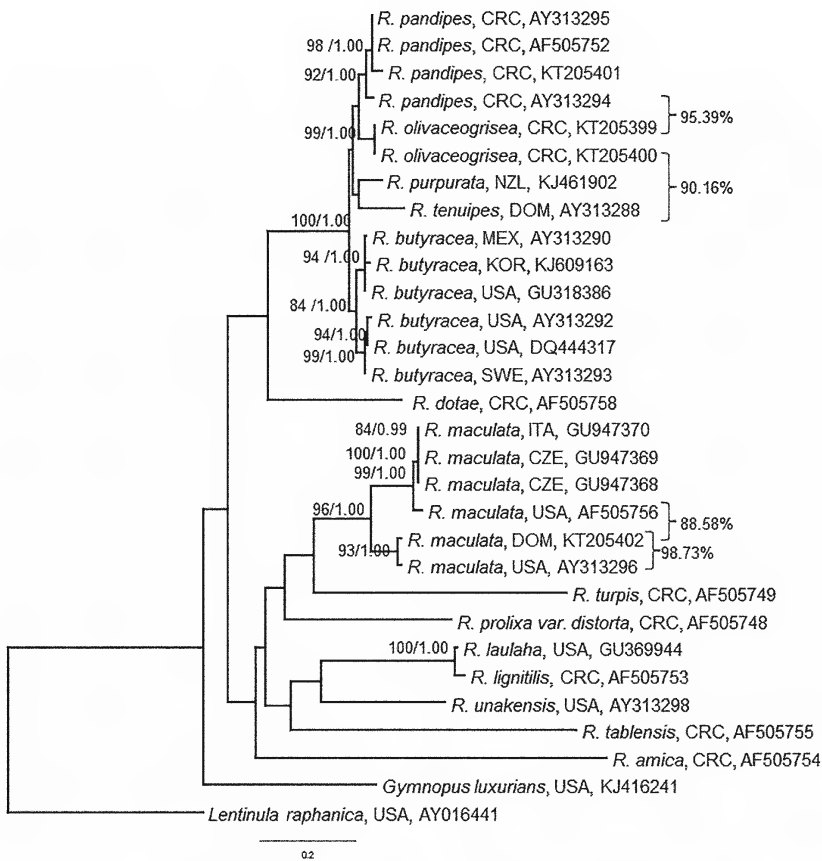


PLATE 1. ITS maximum likelihood phylogeny for neotropical *Rhodocollybia* species. Branch support is presented as: “bootstrap >80%”/“posterior probability”. Numbers after braces are percent identity based on distance matrix. Scale bar is for number of substitutions per site.

weakly supported. This sequence was uploaded in GenBank as *R. aff. pandipes* (Mata et al. 2004) and its phylogenetic placement was similar as in this study. A sequence obtained from a Dominican Republic collection is 98.73% identical to *R. maculata* from Oregon, USA. Although there are two subclades within *R. maculata*, there is not enough data to draw vicariance conclusions, mainly due to insufficient sampling. A newly generated sequence for *R. pandipes* from the Central Volcanic Range in Costa Rica is added in the phylogeny to document range extension.

Taxonomy

Rhodocollybia olivaceogrisea J.L. Mata & Ovrebo, sp. nov.

PLATE 2

MYCOBANK MB 812967

Differs from other *Rhodocollybia* by its green to grayish colored pilei and from *R. pandipes* by its olive to grayish stipes.

TYPE: Costa Rica, Province Cartago, County Oreamuno, District Potrero Cerrado, vicinity of San Juan Chicuá, at Volcán Irazú National Park, Sector Prusia, along El Roble Trail, 9°57.92'N 83°52.29'W, 2950 m a.s.l., 5.Aug.2014, col. J.L. Mata JLM 2175 (Holotype, USAM-F00608; isotype, USJ; GenBank KT205399).

ETYMOLOGY: from Latin *olivaceus* for olive and *griseus* for gray.

PILEUS (10–)35–45(–50) mm broad, broadly convex, to becoming almost plane, shallowly umbonate; surface glabrous, dull or shiny, waxy or greasy to the touch, when young greenish gray [3E3, 2E2, 3E2], olive brown [3C3] with grayish hues, remaining dark olive [3F6] at umbo, grayish olive [3D3] when hygrophanous, losing greenish color with age to drab grayish yellow [90 gy.y–91 d.gy.y]; margin decurved, finely crenulated, pale, translucent. FLESH 2–4 mm thick at center, translucent, watery gray, off-white when hygrophanous. Odor pleasant; taste nondescript. LAMELLAE adnexed, deeply adnexed to free, close (1–2/mm), 1.4–5 mm broad, white when young, in age becoming off-white to cream [2A2–3A2], not discoloring; margin entire or uneven to irregularly wavy (not serrate); two tiers of lamellulae of different lengths. STIPE (40–)80–100 × 4–7 mm at mid stipe, central, ± equal to enlarged gradually towards base and tapered towards apex, ± flexing; surface glabrous, not ridged, drab olive above, ± concolorous to pileus, and becoming olive brown, like pileus, or grayish buff at base; base rounded; consistency very fragile; interior hollow. Whitish mycelium scattered on stipe base.

BASIDIOSPORES 5.5–9 × 3.5–4.5 µm ($n = 40$, $x = 7.2 \times 3.8$ µm, $Q = 1.63$ – 2.33 $Qx = 1.88$), ellipsoid in side view and profile, smooth, dextrinoid; wall thin. PILEIPELLIS a cutis, composed of radially oriented hyphae, 2–6 µm diam, with occasional clusters of semi-erect terminal hyphae, 30–40 × 2–4 µm, mostly with obtuse endings, some with lobes. PILEUS TRAMA composed of radially oriented, interwoven hyphae, 8–20 µm, hyaline, thin-walled; refractive hyphae, 4–6 µm, occasionally present. LAMELLAR TRAMA regular, hyphae 4–16 µm diam, ± interwoven, thin-walled. HYMENIUM composed of basidioles, 15–20 × 3–6 µm, and tetra-sterigmate basidia, 23–30 × 6–8 µm, mostly club-shaped. Pleurocystidia absent. Lamellar margin sterile. CHEILOCYSTIDIA, 20–35 × 5–9 µm, club-shaped to cylindrical, with obtuse apex, frequently with diverticula; abundant. STIPE CUTIS and trama parallel; epicutis hyphae 2–4 µm in diam, context hyphae 4–16 µm in diam, thin-walled. Clamp connections present in all hyphae.

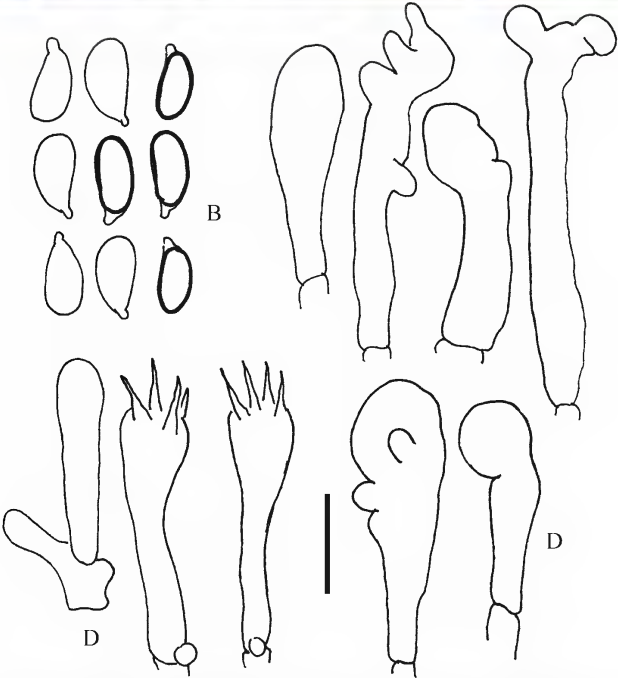


PLATE 2. *Rhodocollybia olivaceo-grisea*. A) basidiomata (JLM 2175); B) basidiospores; C) basidia and basidioles; D) cheilocystidia. Scale bar: B–D = 10 μ m.

ECOLOGY clustered or scattered, some in cespitose bundles, in thick leaf litter layer, under oak.

ADDITIONAL SPECIMENS EXAMINED: COSTA RICA, PROVINCE SAN JOSÉ, County Dota, vicinity of San Gerardo, along road to San Gerardo, 500 m. S. of Interamerican Highway, Km 80, 9°36'13"N 84°47'26"W, 2.Jun.2004, col. C Ovrebo CLO 4368 (NY, USJ).

COMMENTARY— This species resembles *R. butyracea* in the smooth and greasy aspect of the pileus, the swollen stipe, and spore dimensions. But it differs in its gray-olive to brown-olive colors, and in Costa Rica no other *Rhodocollybia* has these field characters. Its closest resemblance is to its Costa Rican sister taxon *R. pandipes*, which is easily distinguished by its bent stipe that soon develops reddish-colors. A tawny olive coloration is reported in pilei of North American *R. lentinoides* (Peck) Halling, but its lamellae become distinctly serrated in time (Halling 2009). The phylogenetically related *R. tenuipes* can be distinguished from *R. olivaceogrisea* by its overall brown pileus colors and a slender stipe that does not develop a strongly swollen base. Basidiomata of *R. olivaceogrisea* do not develop any spots, a field character reported for *R. turpis*, *R. dotae*, and *R. laulaha* [= *R. lignitilis*] in Costa Rica. Its dextrinoid spores set it apart from *R. amica* and *R. tablensis* (Mata et al. 2004).

Rhodocollybia tenuipes J.L. Mata, T.J. Baroni & K.W. Hughes, **sp. nov.** PLATE 3

MYCOBANK MB 812968

Differs from *R. butyracea* by its slender and straight stipe with a striate surface, its less red pileus colors, and its longer spores.

TYPE: Dominican Republic, Province Santiago, Las Placetas, near Los Montones Arribas, vicinity of San José de los Matas, 19°14'N 70°53'W, 8.Jan.2003, col. EA Grand TFB 11707 (Holotype, TENN 59546; GenBank AY313288).

ETYMOLOGY: from Latin *tenuis* for thin, and *pedes* for foot.

PILEUS 10–65 mm diam, convex to broadly convex, becoming broadly campanulate or plane and then uplifted, umbonate, sometimes slightly depressed at disc; surface glabrous, smooth, slightly hygrophonous, burnt sienna [7D8], dull red-brown [5–6D5], cinnamon brown [6E6–7], paler on some [near 5C5–6]; margin curved to uplifted or undulating, incised, cream. FLESH watery cinnamon to cream, thin. Odor none or pungent-spicy, taste unpleasant, bitter. LAMELLAE adnate to adnexed, crowded, 1–4 mm broad, light orange [5A3], cream [5A2], pale cream [4A3] or creamy tan [4A3–5B3], now cream [3A5–4A4]; margin entire, even, concolorous. STIPE 30–80 × 3–9 mm, central, equal to flattened in some parts, somewhat twisted; surface glabrous to pubescent, with striations running entire length, light orange [5A3], pale cinnamon [5C4–5B3], whitish near apex; base slightly swollen in some; interior hollow.

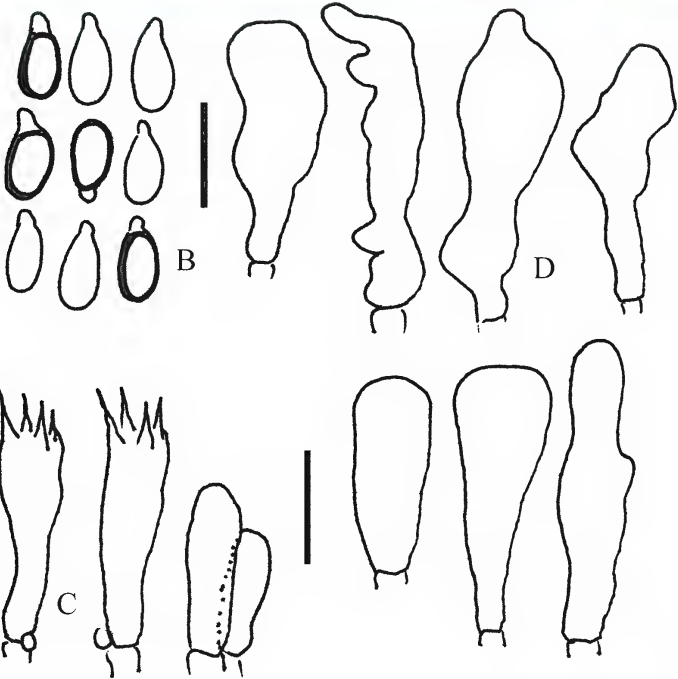


PLATE 3. *Rhodocollybia tenuipes*. A) basidiomata (TJB 8803); B) basidiospores; C) basidia and basidioles; D) cheilocystidia. Scale bars: A = 10 mm; B–D = 10 μ m

BASIDIOSPORES in mass cream [4A2–3]; $5.5\text{--}8.8 \times 3\text{--}4.4\ \mu\text{m}$ ($n = 4/70$, $x = 6.8 \times 3.4\ \mu\text{m}$, $Q = 1.64\text{--}2.33$, $Qx = 2.00$), lacrymoid in side view, ellipsoid in profile, hyaline, inamyloid or dextrinoid (pale reddish-brown to red-brown); wall smooth, thin to slightly thickened in dextrinoid spores, retracted endosporium in some. **PILEIPELLIS** a simple cutis; hyphae $\leq 5\ \mu\text{m}$ diam, cylindrical, repent, radially oriented, somewhat interwoven and gelatinized; wall thin. **PILEUS TRAMA** composed of interwoven hyphae, $\leq 20\ \mu\text{m}$ diam; refractive hyphae present. **LAMELLAR TRAMA** irregular, hyphae $\leq 14\text{--}20\ \mu\text{m}$ diam, long and cylindrical to short and barrel-shaped. **HYMENIUM** composed of basidia, $21\text{--}26 \times 4\text{--}6\ \mu\text{m}$, clavate, four-sterigmate, some dextrinoid; and basidioles $16\text{--}24 \times 3\text{--}5\ \mu\text{m}$, clavate. Pleurocystidia absent. Lamellar margin sterile. **CHEILOCYSTIDIA** $20\text{--}32 \times 4\text{--}7\ \mu\text{m}$, clavate to broadly clavate, or slender, some flexuous, apically obtuse, nodulose to lobed; abundant; wall thin. **STIPE CUTIS** and trama parallel; hyphae of epicutis, $2\text{--}6\ \mu\text{m}$ diam, loosely interwoven, forming a mat, with terminal cells $\leq 50 \times 5\ \mu\text{m}$, long clavate, some lobed or diverticulate, mostly prostrate, some semi-erect; hyphae of trama $6\text{--}16\ \mu\text{m}$ diam, cylindrical; wall thin. Hyphae in all tissues inamyloid and with clamp connections present.

ECOLOGY cespitose on soil, well-decayed wood (like loam); in *Pinus* vegetation.

ADDITIONAL SPECIMENS EXAMINED: DOMINICAN REPUBLIC, PROVINCE LA VEGA, above Manabao, Cruz farm, 21.Nov.1999, coll. T Armstrong TA 265 (CORT 13982); PROVINCE SAN PEDRO DE MACORIS, Corrizal Cordillera, $18^{\circ}52'43''\text{N}$ $69^{\circ}37'26''\text{W}$, 14.Jan.2003, col. EA Grand, TFB 11725 (TENN 59554). PUERTO RICO, MUNICIPALITY LUQUILLO, Ridge above Sabana chicken farm, $18^{\circ}21'03''\text{N}$ $65^{\circ}43'50''\text{W}$, 8.Jun.1998, col. TJ Baroni TJB 8803 (CORT 13983).

COMMENTARY—Fruiting bodies of *R. tenuipes* are similar to *R. butyracea* in color but have slender stipes. Spore dimensions are similar to American reports for *R. butyracea* (Halling 2009) but larger than those cited for European specimens (Antonín & Noordeloos 2010). Basidiomata of *R. tenuipes* lack the bulbous bent stipe base so characteristic of *R. pandipes*, and colors are more reddish-brown than are seen for *R. pandipes*. However, the spore and cystidia dimensions are similar to *R. pandipes*. *Rhodocollybia olivaceogrisea* differs from *R. tenuipes* by its overall olive gray-colored basidiomata. *Rhodocollybia tenuipes* was collected under *Pinus*, a plant genus not indigenous to Costa Rica. DNA extraction from vouchers TA 265 and TJB 8803 (and subsequent PCR intended to obtain a more robust *R. tenuipes* clade) was unsuccessful.

Rhodocollybia maculata (Alb. & Schwein.) Singer, Schweiz. Z. Pilzk. 17: 71. 1939.

Basidiomata of *Rhodocollybia maculata* from the Dominican Republic have pilei that become scrobiculate and cream-colored, lamellae that are

adnexed, crowded, and cream-colored, and stipes with a slightly swollen and bent base, collected under pine duff, or near roots of *Pinus occidentalis*. Micromorphological characters are typical of *R. maculata*, with abundant cheilocystidia $12\text{--}40 \times 4\text{--}8 \mu\text{m}$, clavate to somewhat lobed, or slender and flexuous, apically clavate, furcate or mucronate. Basidiospores $5.2\text{--}6.8 \times 3.2\text{--}4.4 \mu\text{m}$ ($n = 3/55$, $x = 6.2 \times 3.9 \mu\text{m}$, $Q = 1.40\text{--}1.89$, $Qx = 1.56$), short lacrymoid in side view, broadly ellipsoid in profile view, hyaline, inamyloid or dextrinoid (rosy brown to pale orange-brown); wall smooth, thin to slightly thickened in dextrinoid spores.

SPECIMENS EXAMINED: DOMINICAN REPUBLIC, PROVINCE SANTIAGO, Las Placetas, near Bao River, $19^{\circ}14'N$ $70^{\circ}53'W$, 13.Jan.2003, col. EA Grand TFB 11720 (TENN 59804); Ebano Verde, trail from Arroyazo to Loma de la Sal, $19^{\circ}03'03''N$ $70^{\circ}32'46''W$, 31.Aug.2003, col. O Perdomo, M Quirico, A Marmolejo, R Concepción MQ 209 (DR 3020), MQ 212 (DR3021).

This represents a first report of *Rhodocollybia maculata* for Dominican Republic.

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Two new *Puccinia* species on *Melica* (Poaceae) from USA

MEHRDAD ABBASI* & MARY CATHERINE AIME

Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

* CORRESPONDENCE TO: mabbasi@purdue.edu

ABSTRACT—During a study of herbarium specimens of graminicolous rust fungi from the USA deposited in the Arthur Fungarium (PUR), two previously undescribed species infecting *Melica* spp. were encountered. Described as *Puccinia melicae-porteri* sp. nov. and *P. goldsbroughii* sp. nov., they are compared to similar rust fungi on *Melica* spp. from around the world. A key to the fourteen *Puccinia* species on *Melica* spp. is provided.

KEY WORDS—mycobiota, *Pucciniales*, North America

Introduction

The genus *Melica* (Poaceae trib. *Meliceae*) comprises about 80 species worldwide (Clayton & Renvoize 1986). Members of this genus are host to 12 *Puccinia* species. Cummins (1971) reported eight *Puccinia* species on *Melica*: *P. trebouxii* Syd. & P. Syd., *P. graminis* Pers., *P. coronata* Corda s. lat., *P. paradoxa* Ricker, *P. brachypodii* G.H. Otth s. lat., *P. schedonnardi* Kellerm. & Swingle, *P. poarum* Nielsen, and *P. abramoviana* Lavrov. Four additional species have subsequently been reported on *Melica*: *Puccinia pygmaea* Erikss., *P. montanensis* Ellis, *P. persistens* Plowr., and *P. recondita* Dietel & Holw. (Arthur 1934, Tranzschel 1939, Lindquist 1982, Azbukina 1984, French 1989). Index of Fungi (1970–2011) and Farr & Rossman (2015) cite no reports of other *Puccinia* species on *Melica*. As part of a planned study of graminicolous rust fungi in the USA, specimens of *P. schedonnardi* sensu Cummins (1971) on *Melica* spp. deposited in the Arthur Fungarium (PUR) were revised. Our research revealed two species that are morphologically distinct from all previously reported *Puccinia* species on *Melica* and which we describe here.

Materials & methods

Herbarium specimens from PUR were used for this study. Microscopic measurements and photomicrographs were made from material mounted in lactic acid in glycerol (Kirk et al. 2008). At least 30 spores were measured from each specimen. Photomicrographs were taken with a Nikon Digital camera mounted on a Nikon Eclipse 80i microscope. Terminology follows Cummins & Hiratsuka (2003). Spore stages are indicated by conventional symbols: II = uredinia; III = telia.

Taxonomy

Puccinia melicae-porteri M. Abbasi & Aime, **sp. nov.**

PL. 1

MYCOBANK MB 814388

Differs from other *Puccinia* spp. on *Melica* by frequent teliospores with a conical apex or a beak-like projection (tapering rostrum).

TYPE: USA, Colorado, Manitou, Garden of the Gods, on *Melica porteri* Scribn., 18 Oct. 1919, leg. Bethel, II+III (**Holotype**, PUR 21317, as *Puccinia schedonnardi*).

ETYMOLOGY: Named after its host species, *Melica porteri*.

Uredinia on stem and on adaxial leaf surface, elliptic or oblong, golden brown; urediniospores ellipsoid or more or less globoid, $25.6\text{--}31.2 \times 22.4\text{--}25.6\text{ }\mu\text{m}$, wall pale brown, $1.6\text{--}2\text{ }\mu\text{m}$ thick, echinulate, with 7–8 scattered germ pores with slight to moderate internal ring and small pore cap. Telia mostly on the stem (occasionally on adaxial leaf surface), narrowly oblong or linear $\leq 2\text{ cm}$ long, exposed, semi-compact, and blackish, mostly surrounded by ruptured epidermis; teliospores variable in shape, clavoid, oblong-clavoid or fusiform, slightly constricted at septum, rounded, truncate, narrowly conical or with one or (rarely) two beak-like projections (tapering rostrum) at apex, $35.2\text{--}61 \times 15.2\text{--}27.2\text{ }\mu\text{m}$, walls smooth, chestnut brown at apex, lighter below, $1.5\text{--}2.5\text{ }\mu\text{m}$ thick at sides, $\leq 10.5\text{ }\mu\text{m}$ thick at apex ($\leq 20\text{ }\mu\text{m}$ in spores with conical apex), pedicels thick-walled, $\leq 49.5\text{ }\mu\text{m}$ long. Mesospores present.

ADDITIONAL SPECIMENS EXAMINED [all labelled as *Puccinia schedonnardi*]:

On *Melica porteri*: USA, COLORADO, Manitou, Oct. 1919, E. Bethel, III (PUR 21319); Manitou, Garden of the Gods, Glen Eyrie, 27 Sept. 1919, Bethel, II+III (PUR 21318).

The above combination of morphological features distinguishes *P. melicae-porteri* from all previously described *Puccinia* species on *Melica* spp. (Cummins 1971, Farr & Rossman 2015). The closest species to *P. melicae-porteri* is *P. neocoronata*, a rust originally described on *Piptochaetium* and *Stipa* (both from *Poaceae* trib. *Stipeae*). In addition to the host, *Puccinia melicae-porteri* differs from *P. neocoronata* in having mostly caulicolous telia, more morphologically diverse teliospores, and smaller urediniospores with paler walls.



PLATE 1. *Puccinia melicae-porteri* (PUR 21317, holotype): urediniospores and teliospores; teliospore apex truncate, rounded, conical or with beak-like projection (arrows). Scale bars = 10 µm.

Puccinia goldsbroughii M. Abbasi & Aime, sp. nov.

Pl. 2

MYCOBANK MB 814389

Differs from *Puccinia trebouxii* in its smaller teliospores and thin-walled urediniospores with scattered or bizonate germ pores.

TYPE: USA, Missouri, south of Pierpont, Missouri, on *Melica nitens* (Scribn.) Nutt. ex Piper, 24 Jun. 1933, leg. W.E. Maneval, II+III (Holotype, PUR 47968, as *Puccinia schedonnardi*).

ETYMOLOGY: After Prof. Peter Goldsbrough, Department Head of Botany and Plant Pathology, in recognition for his support of the Arthur Fungarium.

Uredinia cinnamon-brown, oblong, amphigenous, mostly on the adaxial leaf surface and stems, sometimes arranged in short rows, first covered by epidermis and later pulverulent, ruptured epidermis around sori is conspicuous; urediniospores more or less globoid, ellipsoid or obovoid, $25.6\text{--}32 \times 23.2\text{--}28\text{ }\mu\text{m}$, wall $1.6\text{--}2\text{ }\mu\text{m}$ thick, densely echinulate, pale-brown, germ pores 6–8, scattered or bizonate with small pore cap. Telia oblong or linear, mostly on adaxial leaf surface and also on the stem, first covered by epidermis, later exposed, semi-compact, blackish brown with a shining appearance, teliospores mostly obovoid-clavoid or oblong, $(28\text{--})32\text{--}44\text{--}(49.6) \times 17.6\text{--}26.4\text{ }\mu\text{m}$, rounded, truncate, acuminate or obliquely acuminate at apex and generally tapering downwards, wall smooth, $2\text{--}3.5\text{ }\mu\text{m}$ at sides, and $\leq 12\text{ }\mu\text{m}$ at apex, clear chestnut brown, lighter towards the base and at apex especially in acuminate spores, pedicels short ($\leq 35\text{ }\mu\text{m}$), mostly shorter than spore length, dark brown below the attachment point to the spore, pale or yellowish brown downward. Mesospores present, common in some telia.

ADDITIONAL SPECIMENS EXAMINED [all labelled as *Puccinia schedonnardi*]:

On *Melica* sp.: USA, INDIANA, Lafayette, 29 July 1899, Wm. Stuart, II+III (PUR 21315); 18 July 1906, J.C. Arthur, II+III (PUR 21347); Lafayette, Elston, 31 Dec. 1896, Wm. Stuart, III (PUR 21348); 22 Oct. 1899, Stuart, II+III (PUR 21314); Lafayette, near Elston, 14 Oct. 1912, J.C. Arthur, II+III (PUR 21372); Lafayette, Bayles Mills, 3 Sept. 1915, J.C. Arthur & H.S. Jackson, II+III (PUR 21360).

On *Melica nitens*: USA, INDIANA, Lafayette, Bayles Mills, 13 May 1903, J.C. Arthur, II+III [III spores mostly germinated] (PUR 21313); 29 May 1903, J.C. Arthur, II (PUR 21312); MISSISSIPPI, Lumberton, 12 Aug. 1921, L.E. Miles, II+III (PUR 45590).

The above combination of morphological features distinguishes *P. goldsbroughii* from all previously reported *Puccinia* species on *Melica* (Cummins 1971, Farr & Rossman 2015). *Puccinia trebouxii*, which was originally described from Samarkand (Uzbekistan), is the morphologically closest species to *P. goldsbroughii*, but differs from *P. goldsbroughii* in its wider teliospores ($24\text{--}36\text{ }\mu\text{m}$) and thick-walled ($\leq 4\text{ }\mu\text{m}$ thick) urediniospores with occasionally equatorial germ pores. On one of the *P. goldsbroughii* paratypes (PUR 21313) teliospores had mostly germinated in telia, but this phenomenon was not observed on other examined material, including the holotype.

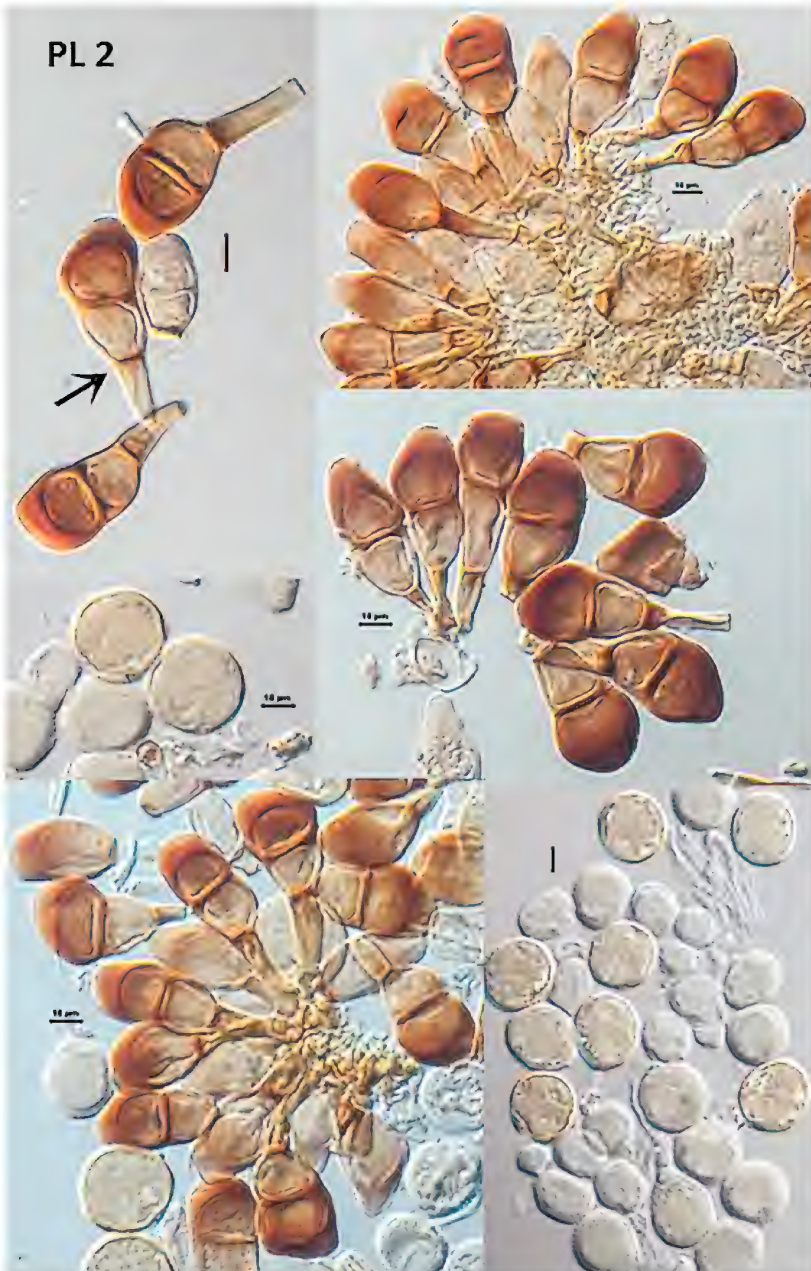


PLATE 2. *Puccinia goldsbroughii* (PUR 47968, holotype): urediniospores, teliospores, and mesospores; arrow shows dark-brown part of pedicel below attachment point. Scale bars = 10 μm.

Key to the 14 *Puccinia* species on *Melica*

1. Teliospores verrucose *P. paradoxica*
1. Teliospores smooth 2
2. Telia exposed 3
2. Telia covered 7
3. Teliospores with apical beak-like projection *P. melicae-porteri*
3. Teliospores without projection 4
4. Urediniospore pores exclusively equatorial *P. graminis*
4. Urediniospore pores scattered, occasionally equatorial 5
5. Teliospores mostly broadly ellipsoid, broadly obovoid or more or less globoid
..... *P. schedonnardi* s. str.
5. Teliospores mostly obovoid-clavoid or oblong 6
6. Teliospores 24–36 µm wide *P. trebouxii*
6. Teliospores 17.6–26.4 µm wide *P. goldsbroughii*
7. Teliospores with apical digitations *P. coronata* s. lat.
7. Teliospores without apical digitations 8
8. Uredinia paraphysate 9
8. Uredinia aparamphysate 11
9. Paraphyses thick-walled *P. brachypodii* s. lat.
9. Paraphyses uniformly thin walled 10
10. Teliospores mostly 22–32 µm wide *P. montanensis*
10. Teliospores mostly 17–22 µm wide *P. pygmaea*
11. Urediniospores verrucose *P. abramoviana*
11. Urediniospores echinulate 12
12. Urediniospore wall colorless or pale yellowish *P. poarum*
12. Urediniospore wall brownish 13
13. Teliospores mostly 35–70 µm long *P. recondita* s. str.
13. Teliospores mostly 34–46 µm long *P. persistens*

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Regional annotated mycobiotas new to the Mycotaxon website

ABSTRACT—Mycotaxon is pleased to announce three new species distribution lists to our ‘web-list’ page covering *Glomeromycota* in Brazil (by Jobim, Oliveira & Goto), macromycetes within *Cistaceae*-dominated ecosystems in Cyprus (by Loizides), and macrofungi of Muğla province in Turkey (by Güngör, Solak, Allı, Işıloğlu & Kalmış). This brings to 123 the number of free access mycobiotas now available on the Mycotaxon website:
<http://www.mycotaxon.com/resources/weblists.html>

SOUTH AMERICA

Brazil

KHADIJA JOBIM, BRUNA IOHANNA SANTOS OLIVEIRA & BRUNO TOMIO GOTO.
Checklist of the *Glomeromycota* in the Brazilian Savanna. 13 p.

ABSTRACT—The Brazilian savanna (Cerrado) was the first Brazilian biome to be surveyed for arbuscular mycorrhizal fungi (AMF) and currently comprises the third Brazilian biome in species representation. This paper provides a checklist of arbuscular mycorrhizal fungi (AMF) in the Cerrado. A total of 92 species of AMF have been found in the Brazilian Cerrado over three decades of work conducted in this biome. The results characterize the Cerrado as an important AMF reservoir and show that rupestrian fields, one of several physiognomies of the cerrado, are biologically promising

EUROPE

Cyprus

MICHAEL LOIZIDES. Macromycetes within *Cistaceae*-dominated ecosystems in Cyprus. 33 p.

ABSTRACT—An inventory of macromycetes associated with *Cistaceae* plants, including *Cistus*, *Helianthemum*, *Tuberaria* and *Fumana* species in Cyprus is presented, following a ten-year survey between 2007 and 2016. One-hundred-and-twenty-seven taxa are identified, sixty-five of which are reported for the first time from Cyprus. Of these, some recently described or rarely reported species are noteworthy,

such as *Agaricus iesu-et-marthae*, *Astraeus telleriae*, *Fomitiporia rosmarini*, *Gymnopus bisporus*, *Lepiota farinolens*, *Lepiota locquinii*, *Ombrophila rivulorum*, *Peziza muscicola*, *Pholiota gallica*, *Tremella dactylobasidia* and *Xeromphalina cornui*. The taxonomical problems associated with a number of insufficiently clarified taxa, such as *Clitocybe font-queri*, *Cortinarius caligatus*, *Leccinellum corsicum*, *Lyophyllum fumosum*, *Peziza moseri*, *Peziza subviolacea*, *Plectania zugazae* and *Terfezia aphroditis* are discussed, and the role of *Cistus* communities in Mediterranean ecosystems is evaluated, particularly in view of accelerated climate changes. Selected imagery and notes about the fruiting season, host-plant, altitude and estimated abundance are provided.

MID-EAST

Turkey

HALİL GÜNGÖR, MEHMET HALİL SOLAK, HAKAN ALLI, MUSTAFA IŞILOĞLU, ERBİL KALMIŞ.
Contributions to the macrofungal diversity of Muğla province (Turkey). 26 p.

ABSTRACT—This study was conducted on specimens of macrofungi collected from Muğla province between 2005-2011. After field and laboratory studies, 211 taxa belonging to 47 families and 2 classes were identified. Five of them—*Cortinarius biformis*, *Cortinarius percomis*, *Cortinarius renidens*, *Geastrum lageniforme*, *Hebeloma aestivale*—represent new records for Turkey.

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BOOK REVIEWS AND NOTICES*

ABSTRACT—Books reviewed include: FIELD GUIDES—The essential guide to Rocky Mountain mushrooms (Cripps, Evenson & Kuo 2016); LICHENS—Keys to lichens of North America (Brodo 2016).

FIELD GUIDES

The essential guide to Rocky Mountain mushrooms by habitat. By C.L. Cripps, V.S. Evenson & M. Kuo. 2016. University of Illinois Press, 1325 South Oak Street, Champaign, IL 61820-6903 <www.press.uillinois.edu>. 272 p, 372 colour photos, ISBN 978-0-252-08146-0 US \$29.95 (soft cover); ISBN 978-0-252-03996-6 \$ 95.00 (hard cover).

The mushrooms of the Rocky Mountains from Montana to Colorado (USA) southwards are covered in this lavishly illustrated book.

Unlike the usual guide, this book is organized by habitat, from the prairies of the foothills through the extent of montane Ponderosa pine and subalpine Spruce-Fir forests to the alpine high altitude vegetation. Each habitat is introduced with a description of the main players, which includes plants, animals, and fungi, followed by descriptions and photos of the characteristic mushrooms of that particular habitat. The book is organized like a walk up the mountains, but has also a chapter on burned ground and on snowbanks, with their characteristic mycoflora. Snowbank fungi are unique to western North America, and certainly deserve their own place in this book.

* Book reviews or books for consideration for coverage in this column should be sent to the *Editor-in-Chief* at editor@mycotaxon.com or 6720 NW Skyline, Portland OR 97229 USA.

The only other North American guide organized by habitat that I know of is the one on Hawaiian fungi (Hemmes & Desjardin 2002). Special for the Rocky Mountain book is the attention to organisms other than fungi.

Unlike the usual guides, this book does not have an extensive introduction to fungi; one page on making spore prints is placed in the introduction, simple keys are given at the end, and one page is devoted to eating mushrooms.

The index also serves as glossary, with terms explained with a referral to the page where the term is used.

The approach to place the fungi so clearly in their environment—and to show the interconnectedness between all organisms in a certain habitat—is wonderful.

The emphasis is on the more conspicuous and easy to recognize species; one will look in vain for an *Inocybe* species in this book, although many occur in the Rocky Mountains. The descriptions cover macroscopical characters, and the observations give information on similar species of the mountains.

One objection to the habitat approach is that of course some fungal species occur in several habitats; to counter this, the inside of the front cover lists the species per habitat and for each species also indicates in which other habitats it might occur. Still, when one wants to compare *Suillus* species that occur in the pine forests, one has to flip back and forth between habitats.

The quality of the photos bothers me – many photos are very flat, which has probably to do with the printing process. Most photos of the species are good and show all the characters of a species, while others do not include the complete fruitbodies.

Some names still need to be updated – some examples: *Plectania nannfeldtii* should be *Donadinia nigrella* (Carbone et al. 2013), and the more recent genus for *Pachylepyrium*, *Crassisporium* (Matheny et al. 2015), is named in the “Observations” as an option.

The approach presented in this book makes me want to see more books like this, and I hope that the increasing knowledge of the North American mycoflora will make this possible.

Carbone M, Agnello C, Alvarado P. 2013. Phylogenetic studies in the family *Sarcosomataceae* (*Ascomycota*, *Pezizales*). *Ascomycete.org* 5 (1): 1–12.

Hemmes DE, Desjardin DE. 2002. *Mushrooms of Hawai'i. An identification guide*. Ten Speed Press, Berkeley, Toronto.

Matheny PB, Moreau P-A, Vizzini A, Harrower E, De Haan A, Contu M, Curti M, 2015.

Crassisporium and *Romagnesiella*: two new genera of dark-spored *Agaricales*. *Systematics and Biodiversity* 13: 28–41. <http://dx.doi.org/10.1080/14772000.2014.967823>

ELSE C. VELLINGA

861 Keeler Avenue, Berkeley CA 94708 USA
ecvellinga@comcast.net

LICHENS

Keys to lichens of North America: revised and expanded. By I.M. Brodo, with photographs by S.D. Sharnoff & S. Sharnoff and drawings by S. Laurie-Bourque. 2016. Yale University Press, P.O. Box 209040, New Haven, CT 06520-9040. <sales.press@yale.edu> ISBN 978-0-300-19573-6. 424 p., 13 color and 33 b/w illustrations. \$29.95.

When *LICHENS OF NORTH AMERICA* (Brodo et al. 2001) was published, it quickly became a classic. The book gave a thorough introduction into the world of lichens and provided keys to genera and many species. Beautiful color photographs illustrated the treated species, and a number of additional species were mentioned for comparison. All this made it an invaluable contribution to lichenology in North America. Over the years, Brodo has received numerous requests for a separate volume that could be used more practically as teaching material in the lab and field. This newly revised *KEYS TO LICHENS OF NORTH AMERICA* is a response to those requests.

In this treatment, Brodo has greatly expanded and updated the original keys found in *LICHENS OF NORTH AMERICA*. After a brief preface explaining the purpose of the present volume followed by acknowledgements, the *KEYS TO LICHENS OF NORTH AMERICA* dives directly into introductory identification keys to genera and the following major groups: basidiolichens, fruticose lichens, dwarf fruticose lichens, sterile crustose lichens, crustose perithecial lichens, crustose script lichens, crustose disk lichens, squamulose lichens, umbilicate lichens, jelly lichens, orange-yellow-green foliose lichens, and non-umbilicate, non-jelly, non-yellow foliose lichens. All genera as well as many easy-to-identify species are separated in the group keys. Keys to genera and major groups are followed by keys to species level where the genera are treated in alphabetical order. Thus, more advanced lichenologists can turn directly to the genus keys without having to wade through the introductory keys. The original glossary, which contains clarifying drawings by Laurie-Bourque and photographs by the Sharnoffs from *LICHENS OF NORTH AMERICA*, is also included. Finally, references and an index to names of genera and species complete the volume.

The layout is very clean and organized. Although I would prefer an indented key because such a format fosters navigation and retracing earlier steps, the bracket format keeps couplet components together and consumes far less space. The keys are well written and contain abundant detail. For example, a spore is not just “thick-walled” versus “thin-walled” but the key-step contains average measurements that facilitate determination. While specialized terminology

cannot be completely avoided in any scientific discipline, Brodo has limited his use of technical jargon, especially compared to some regional treatments (e.g. Nash et al. 2002, 2004, 2007). These qualities extend this volume's utility to beginners and experts alike. The keys are of course not comprehensive; including all North American taxa would require a volume more than twice as thick, not to mention an enormous amount of work, since only about 42% (2028 species) of the 4881 known species in North America (Esslinger 2015) are treated. However, the most common and prominent species that users will encounter are included. This makes the keys very useful as a first reference, especially as a laboratory resource for university courses and workshops as well as for amateur enthusiasts. Advanced lichenologists will find utility in the general keys to reach genus level before seeking more comprehensive, specialized monographs and regional treatments offering deeper taxonomic resolution. A minor disadvantage of the *KEYS TO LICHENS OF NORTH AMERICA* is that species are not described in detail. However, the volume is intended as a companion for the original *LICHENS OF NORTH AMERICA* where detailed descriptions, color photographs, distribution maps, and comparative notes are provided for many of the mentioned species.

Overall, this volume of keys fully lives up to and complements *LICHENS OF NORTH AMERICA*, and it is an essential identification tool for professional and amateur lichenologists alike. Brodo invites comments, problems, and errors in order to continually improve and update the keys. *KEYS TO LICHENS OF NORTH AMERICA* and potential updates will certainly boost the future of North American lichenology.

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RIKKE REESE NÆSBORG
University of California at Berkeley
Rikke.ReeseNaesborg@gmail.com

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NOMENCLATURAL NOVELTIES AND TYPIFICATIONS

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Agaricus columellatus (Long) R.M. Chapm., V.S. Evenson & S.T. Bates
(lectotypified) [MB809082], p. 105

Anacoronospora J.S. Monteiro, Gusmão & R.F. Castañeda [MB813219], p. 186

Anacoronospora diversiseptata J.S. Monteiro, Gusmão & R.F. Castañeda
[MB813220], p. 186

Annulohypoxylon bogoriense (Höhn.) Lar.N. Vassiljeva, S.L. Stephenson &
K.D. Hyde [MB801406], p. 63

Annulohypoxylon Chiangmaiense Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
[MB801393], p. 64

Annulohypoxylon derelictum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
[MB801394], p. 66

Annulohypoxylon dipterocarpi Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
[MB801395], p. 66

Annulohypoxylon maesaeense Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
[MB801396], p. 67

Annulohypoxylon microbovei Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
[MB801397], p. 68

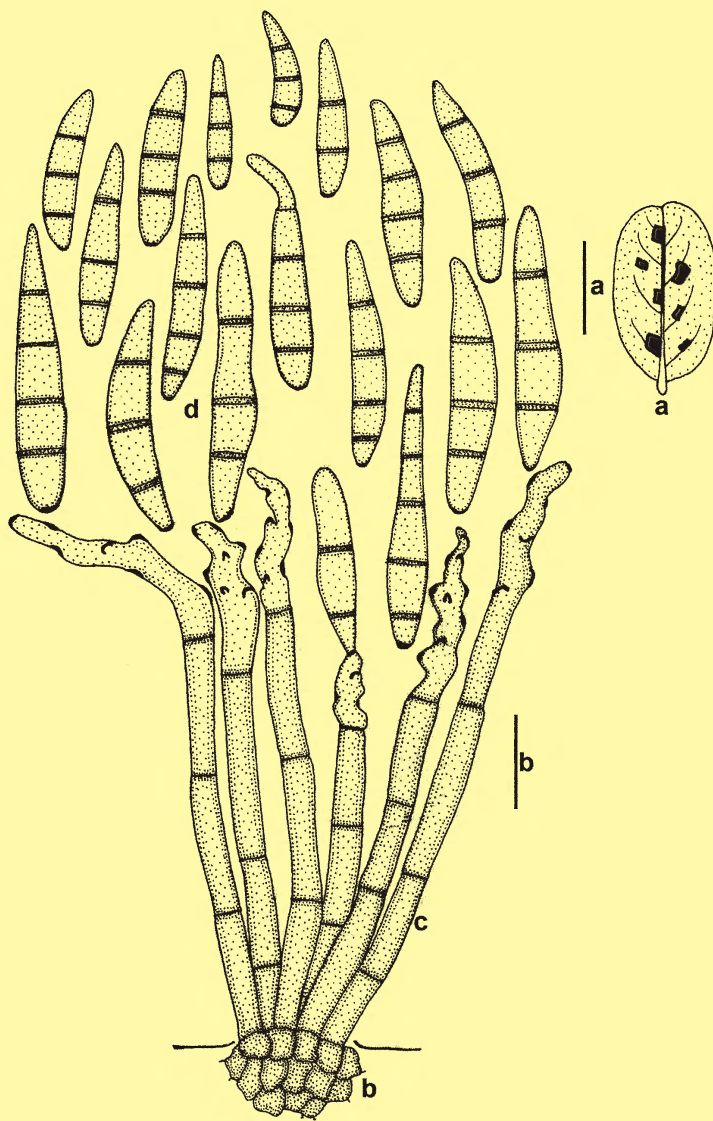
Annulohypoxylon morisuspectum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
[MB801398], p. 70

Annulohypoxylon neglectum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
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[MB801401], p. 72

Annulohypoxylon planodiscum Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde
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- Annulohyphoxylon pseudonitens* Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde [MB801403], p. 74
- Annulohyphoxylon sordidum* Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde [MB801404], p. 75
- Aspergillus similanensis* Dethoup [MB810782], p. 9
- Dictyoaquaphila* J.S. Monteiro, L.B. Conc., M.F.O. Marques, Gusmão & R.F. Castañeda [MB813243], p. 178
- Dictyoaquaphila appendiculata* J.S. Monteiro, L.B. Conc., M.F.O. Marques, Gusmão & R.F. Castañeda [MB813244], p. 178
- Fomitiporia deserticola* Vlasák [MB813967], p. 198
- Fomitiporia fissurata* Vlasák [MB813966], p. 196
- Gibellula clavispora* Z.Q. Liang, Wan H. Chen & Y.F. Han [MB810567], p. 115
- Leptonia newlingii* Largent & Bergemann [MB813886], p. 170
- Leptonia sabulosa* Largent & Bergemann [MB813883], p. 167
- Leptonia subpanniculus* Largent & Bergemann [MB813877], p. 160
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- Mirandina uncinata* Fiuza, J.S. Monteiro, R.F. Castañeda & Gusmão [MB813056], p. 143
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- Pleurothecium bicoloratum* R.F. Castañeda, J.S. Monteiro, & Gusmão [MB813040], p. 146
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- Rhodocollybia olivaceogrisea* J.L. Mata & Ovrebo [MB812967], p. 239
- Rhodocollybia tenuipes* J.L. Mata, T.J. Baroni & K.W. Hughes [MB812968], p. 241
- Sarcogyne saphyniana* A. Abbas, Nurtai & K. Knudsen, p. 136
- Sporidesmiopsis pluriseptata* J.S. Monteiro, Gusmão & R.F. Castañeda [MB813041], p. 150
- Sympodiosynnema* J.W. Xia & X.G. Zhang [MB815649], p. 46
- Sympodiosynnema elegans* J.W. Xia & X.G. Zhang [MB815650], p. 46
- Ustulina pseudozonata* Lar.N. Vassiljeva, S.L. Stephenson & K.D. Hyde [MB801405], p. 80



Passalora caesalpinicola sp. nov.
 (Kumar & Singh— FIG. 2, p. 28)
 SHAMBHU KUMAR, artist